THE PATHOGENESIS AND PRE-OPERATIVE DIAGNOSIS OF INFLAMMATORY ANEURYSMS OF THE AORTA

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ABSTRACT.

Inflammatory abdominal aortic aneurysms (IA) constitute approximately 15% of all abdominal aortic aneurysms. Macroscopically, they are characterised by a thick white rind of fibrous tissue, which may extend in the retroperitoneum to encase and obstruct adjacent hollow organs. This may require major changes and modifications in exposure and operative technique.

Currently, diagnostic accuracy is poor in inflammatory aortic aneurysm disease, and little is known of its pathogenesis. This leads to many such aneurysms remaining unsuspected pre-operatively, and contributes to the increased operative morbidity and mortality of this disease.

A review of the history of vascular surgery and radiology is undertaken, with a particular emphasis on aneurysm disease. Current theories of the ætiology of both simple (SA) and inflammatory aneurysm are discussed. An account is given of the current state of radiological techniques in the diagnosis of inflammatory aneurysm disease, and a brief technical synopsis of magnetic resonance imaging (MRI) is provided.

A retrospective series was studied, comprising 47 patients with IA and 162 patients with SA of matched age and sex distribution. No differences were found in the incidence of diabetes, smoking, symptomatic occlusive vascular disease or hypertension. Pain was slightly more common in the IA group, but weight loss was not. Plasma viscosity was the only measured hæmatological parameter which differed significantly between the groups, being higher in the IA group compared to the other. Biochemical evidence of renal failure was a poor predictor of ureteric involvement in fibrosis.

Pre-operative ultrasound failed to make the diagnosis of IA in all cases subsequently confirmed at operation. Computerised tomography (CT) made the diagnosis in only 13 of 25 cases. All aortic replacements were carried out using the transperitoneal route. In 4 cases of IA however, unexpectedly extensive abdominal inflammatory change required the abdominal incision to be extended as a left eighth rib thoracotomy. 63% of patients with IA required bifurcation grafts rather than tube grafts, the figures being almost exactly reversed for patients with SA.

Operations involving IA took significantly longer, and required more blood and colloid infusion than operations on simple aneurysms.

Operative (30 day) mortality for the IA group was 13%, but only 4.3% for SA.

A further series was prospectively studied comprising 15 patients with IA, 85 with SA and 37 with occlusive disease (OCC). Findings of the retrospective study in risk factors, clinical findings, symptomatology and hæmatological criteria are confirmed.

Peripheral blood from all groups was assayed for a number of biochemical attributes. Higher levels of acute phase proteins and neutrophil elastase were found in the IA group, and initial findings suggest that elastin fragment assay is also higher. Elastase inhibitory activity was also greater in the IA group, although there was no difference in antitrypsin phenotypes.

Radiological diagnosis of inflammatory change, diagnosis of aneurysm level and the visualisation of the renal arteries were all poor using CT and ultrasound. Seen on MRI scans, the walls of IA have a characteristic appearance of concentric layering. This enabled MRI to make the diagnosis of IA in all studied cases. MRI also offered improved visualisation of the upper

extent of aneurysms, and of renal artery origins.

The results of this work support the current theories of ætiology of inflammatory aneurysms. There is evidence of an increase in active elastin breakdown in IA secondary to increased elastase activity rather than any defect in elastase inhibition mechanisms. This suggests a theory of self perpetuation in IA which is discussed.

A previously unreported appearance of inflammatory aneurysm walls has been noted using Magnetic Resonance Imaging. This modality was also found to offer improved definition of the upper limit and the renal artery origins of all aneurysms. This enhanced diagnostic ability led to an improvement in operative mortality.

The work which is described in this thesis is my own. The experimental work included has been carried out by myself, except for the following -

Acute phase protein assays were carried out in the Department of Chemical Pathology, Bristol Royal Infirmary, by Mrs. June Morgan, Senior M.L.S.O. She also carried out all Antitrypsin Phenotyping procedures.

Magnetic Resonance Imaging was carried out by the Radiographic Staff of Frenchay Hospital, Bristol, under the Supervision of Dr. G. G. Hartnell, Consultant Radiologist. Other radiological investigations were performed in Bristol Royal Infirmary's Department of Radiodiagnosis.

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CHAPTER 1

AIMS, INTRODUCTION AND HISTORICAL REVIEW

The aims of this thesis are as follows:

1. To study the differences in presentation, clinical features, diagnosis, operative details, and subsequent outcome in a large retrospective series of inflammatory and non-inflammatory aneurysms of the abdominal aorta.

2. To prospectively study the biochemical and radiological characteristics of groups of inflammatory and non-inflammatory aortic aneurysms. To define differences in these attributes and to discuss their use in diagnosis and treatment, and to try to clarify the nature of the inflammatory process.

INTRODUCTION

Inflammatory aortic aneurysms comprise 2.5 - 23% of all abdominal aortic aneurysms. In common with simple aortic aneurysms, they occur principally in males, but in a population approximately a decade younger. Characteristically, inflammatory aneurysms are covered in a thick white coat of fibrous tissue which may extend laterally into the retroperitoneum to involve other organs, encasing them in fibrosis. Organs which may be so encased include the duodenum, ureters, left renal vein and inferior vena cava. It is this feature which may cause difficulty at operation and require considerable modification of operative technique.

Many inflammatory aneurysms go unsuspected. As a consequence, an appropriate operative approach cannot always be planned. The aneurysm may be deemed inoperable at laparotomy with subsequent fatal consequences.

It is clearly in the best interest of both patient and surgeon to be aware of the presence of inflammatory aneurysm pre-operatively.

Current diagnostic tests for inflammatory aneurysms are unreliable and non-specific. These have been reviewed in a large retrospective series of inflammatory and non-inflammatory aneurysms highlighting differences in presentation, clinical features, investigation, operative details, and subsequent outcome.

Other sections of this thesis relate to a prospectively studied series. They deal with contrasting biochemical and radiological characteristics of inflammatory and non-inflammatory aneurysms and aorto-iliac occlusive disease. These contrasts are presented in relation to the pathogenesis of inflammatory aneurysms, and their possible use in diagnosis is discussed.

For the purposes of this thesis, the following definitions shall apply:

1. Inflammatory aneurysm. An aortic aneurysm over 4 cm in diameter, which presents the white 'sugar-icing' appearance of dense mural fibrosis at operation. Other organs may or may not be involved in this fibrosis. The cut wall is thick, and histologically meets previously published criteria.[1]

2. Simple aneurysm. An aortic aneurysm over 4 cm in diameter, which does not present the typical appearances of (1.) above. There is a thin wall with little mural fibrosis, and no adherence or involvement of adjacent organs.

3. Aorto-iliac occlusive disease. Stenosing or occlusive atheromatous disease affecting the infra-renal aorta, the aortic bifurcation, or the common iliac arteries, presenting with severe lower limb ischæmia.

THE DEVELOPMENT AND EMERGENCE OF ANEURYSM SURGERY

Early Arterial Surgery

Disorders of the blood vessels whether through injury or degeneration often present with physical signs of an easily discernible and frequently dramatic nature. It is not surprising therefore, that details of the treatment of bleeding, and of aneurysmal dilatation of the peripheral arteries are found in the earliest records of surgical intervention.

Vascular surgery probably has its roots in Susruta's use of a ligature on the umbilical cord in 1500 B.C.[2] Ligatures were subsequently described to arrest bleeding by placing them around a limb (Chrysippus of Crudes 340 BC).[3] They were certainly described in Alexandrian times (approximately 200 BC)[4] by Celsus (25-50 BC)[5] and by Archigenes (48-117 AD)[6], but the hæmostatic of choice remained cautery. It was not until Parés rediscovery of the ligature in 1552[7] that the technique came into popular use.

Peripheral Arterial Aneurysms

Aneurysms have been known from at least Egyptian times, the Ebers Papyrus describing what are likely to have been traumatic aneurysms of the extremities.[8] Galen (AD 131-200) was probably the first to formally document aneurysms in his work "De Tumoribus", recognising that they arose from arteries, and were filled with blood.[9]

The treatment of peripheral arterial aneurysms is the foundation upon which modern vascular surgery was built. Such aneurysms were not only readily visible, but surgical access was relatively simple in the pre-anæsthesia era. Aneurysms of the abdominal aorta were first described by Vesalius in the 16th century, but because of the impossibility of surgical treatment, aroused little interest for three hundred years thereafter.[10]

Antyllus (AD 48-117) appears to have described the first technique for the surgical treatment of peripheral aneurysm, tying off the vascular mass at both ends close to the sac, then evacuating the contents.[11,12] His detailed description of the surgical technique acknowledges the folly of treating aneurysms of the neck, axilla or groin by ligation, and comments on the increase in ligature security gained by leaving the aneurysm sac in situ, rather than excising it. This principle was ignored by Aetius in the 6th century, who followed Antyllus' technique, but in addition recommended division of the feeding vessel.[13]

The Antyllian operation of bipolar ligation and incision of the aneurysm persisted as one of the principle procedures for the treatment of peripheral aneurysms until the 18th century.[14]

The next major advance in aneurysm treatment was made by Paré in the second half of the 16th century. In addition to investigating the pathology of aneurysms[15] and proposing syphilis as a cause, he is thought to have been the first to use a single proximal ligature in the treatment of aneurysm. This change in technique appears to have gone largely unnoticed at the time, but clearly precedes its successful use by Anel in 1710.[16]

The treatment of aneurysms remained rooted in the use of a number of broadly similar operations from the time of Antyllus until the middle and late 18th century. Techniques were described using proximal or bipolar ligation, with or without incision of the aneurysm sac and evacuation of its contents.[17] Pressure applied and maintained by bandages was often used as a first line treatment in an attempt to induce thrombosis in the aneurysm. Only if this had no appreciable effect did the surgeon resort to open operation.[18]

Ligation of the aneurysm sac was frequently carried out for advanced disease, where the technical difficulties were considerable, and the sac was often ruptured accidentally by the surgeon. Even if ligation could be achieved without the patient exsanguinating, suppuration of the incised sac or wound regularly led to fatal secondary hæmorrhage. The outcome of aneurysm surgery was so frequently fatal that Percival Pott was of the opinion in 1779 that amputation was the best policy.[19] An improvement in surgical technique was clearly required, and was supplied by the experimental surgeon, John Hunter.

It is surprising that John Hunter is popularly credited with the first use of the single proximal ligature, in view of the availability for some decades of Heisters (1683-1758) account of the technique[20], and its use by Guillemeau and Anel[21]. The chief differences in Hunters technique were:

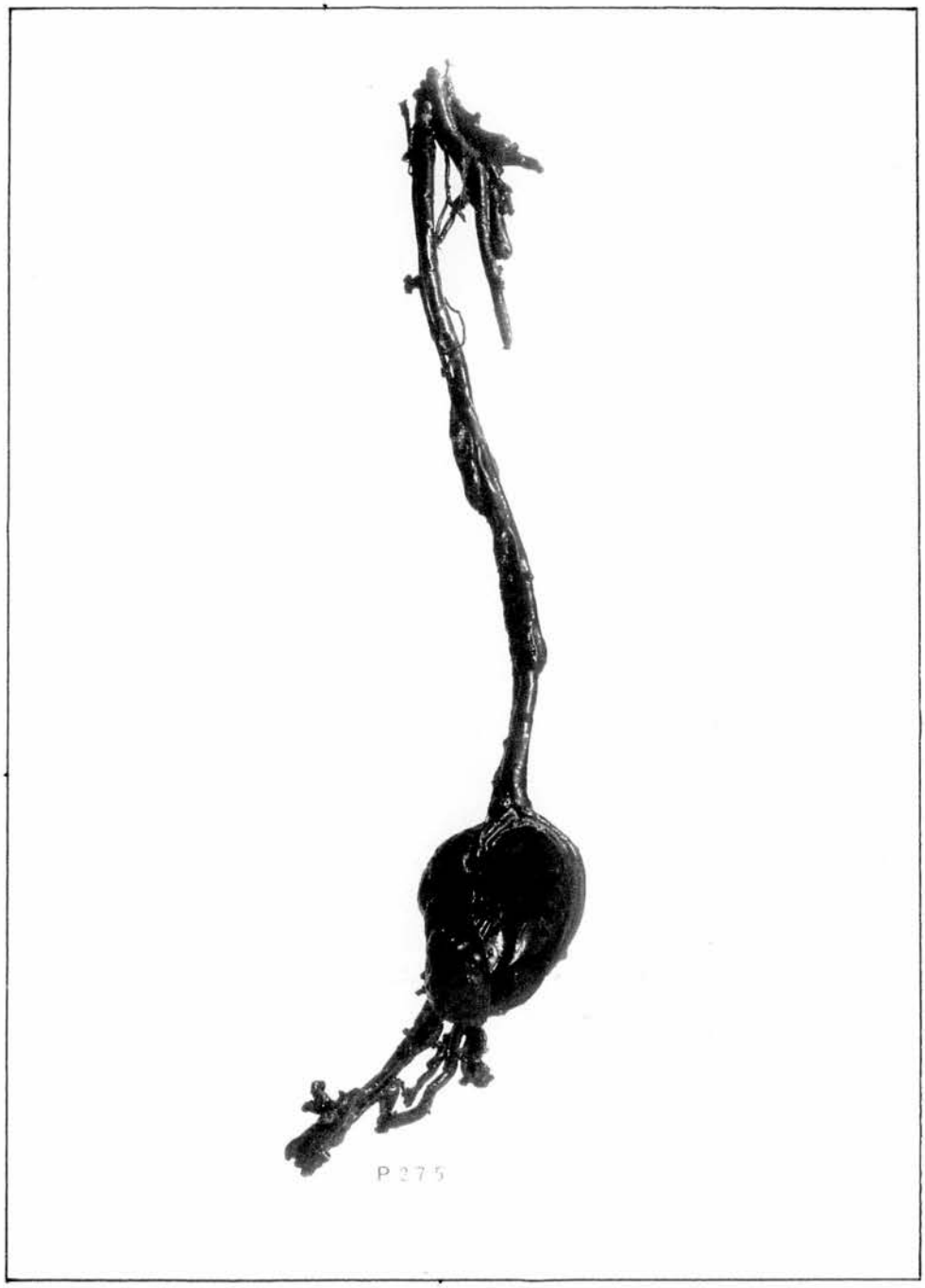
1. The omission of evacuation of the aneurysm sac.
2. Placement of the proximal ligature at a much higher level than had previously been the norm.

Hunter reasoned that infection and fatal hæmorrhage would be reduced if the vessel feeding the aneurysm was ligated at a remote site, through healthy tissue, without disturbing the sac. Following a period of experimentation , the first operation based on his findings was performed by Hunter, in 1785, on a coachman with a popliteal aneurysm. Hunters operation was rapidly adopted by other surgeons, and remained a standard operative treatment for popliteal aneurysm until the early 1950s.[22-24]

Figure 1 (overleaf).

This shows the preserved popliteal aneurysm of the 45 year old coachman upon whom Hunter operated in 1785. The obliterated femoral artery and the femoral vein are preserved in connection with the remains of the aneurysm sac.

The specimen is shown by kind permission of the Hunterian Museum of the Royal College of Surgeons of England. A full account of the case can be found in the Museum catalogue.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Alternatives to ligation: Reconstruction and Grafting

The operation of obliterative endaneurysmorrhaphy was introduced by Matas in 1888.[25] He had performed the Antyllian operation on a young man who had presented with a traumatic brachial artery aneurysm, but noted that bipolar ligation did not reduce the arterial pulsation. He opened the aneurysm sac, to reveal three orifices; the two polar vessels which he had ligated, and a collateral which was still openly bleeding. He oversewed the collateral, then closed the sac. The patient made a good recovery. This obliterative operation was then extended to restorative and reconstructive procedures preserving flow through the artery.[26,27] Although Matas reported good results, the operation did not gain wide acceptance, and was little used.

From the late 19th century onwards, work had been proceeding on the direct suture of blood vessels and on allografting.

By the end of the 19th century, a number of experimental surgeons including Jassinovski[28] and Dörfler[29] had succeeded in direct arterial suture using aseptic technique. These were all lateral sutures. Simultaneously, work was being performed on non-suture techniques of blood vessel anastomosis using a variety of materials.[30] This work was later adapted and developed by Blakemore[31] for use in war casualties, and persists in current surgical technique in the use of non-sutured aortic prostheses.[32] Nevertheless, work continued on the technique of sutured blood vessel anastomosis, and the first successful circular suture of an artery in a human was performed by J.B Murphy in 1896.[33]

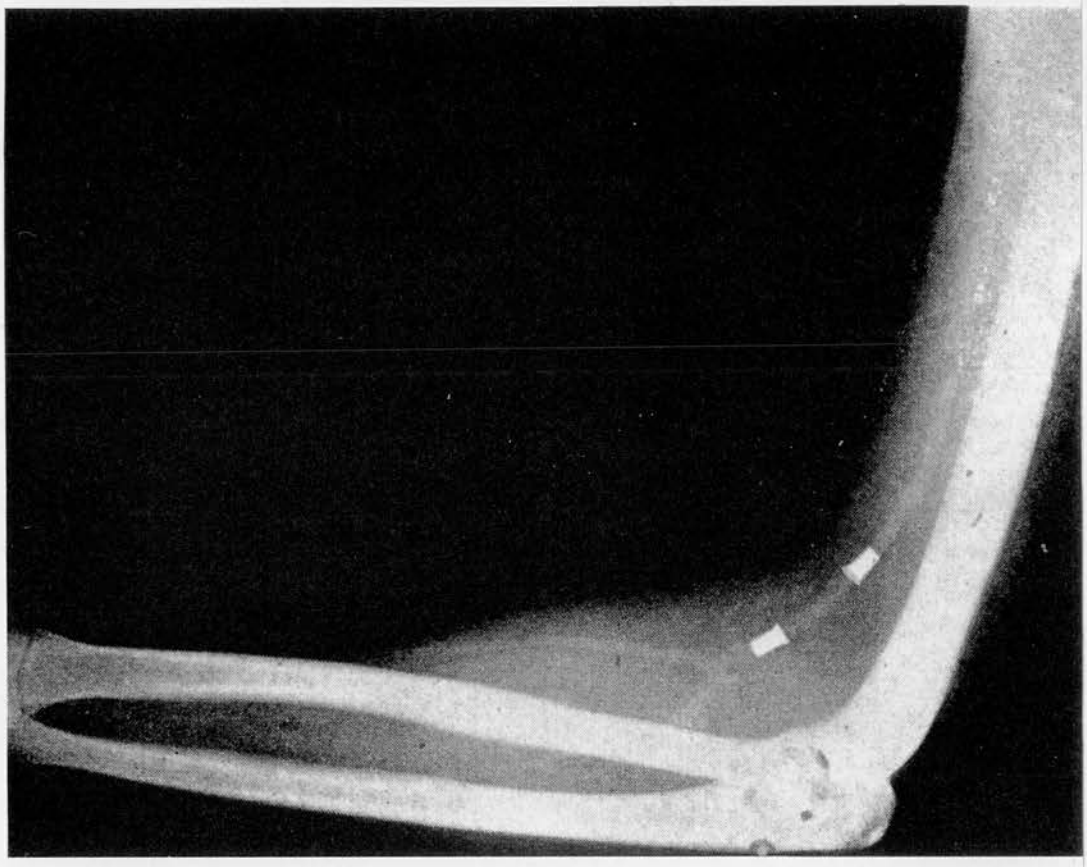


Figure 2a.

Angiogram of Case 2 in Blakemores paper[31] from 1945.The patient was a 15 year old boy whose left arm had been damaged when falling through a plate glass window. There was segmental loss of the brachial artery, which was bridged using the non-suture technique pioneered by Blakemore. The angiogram was performed 34 days after the operation, and shows a Vitallium tube at each end of the vein segment used to bridge the left brachial artery.

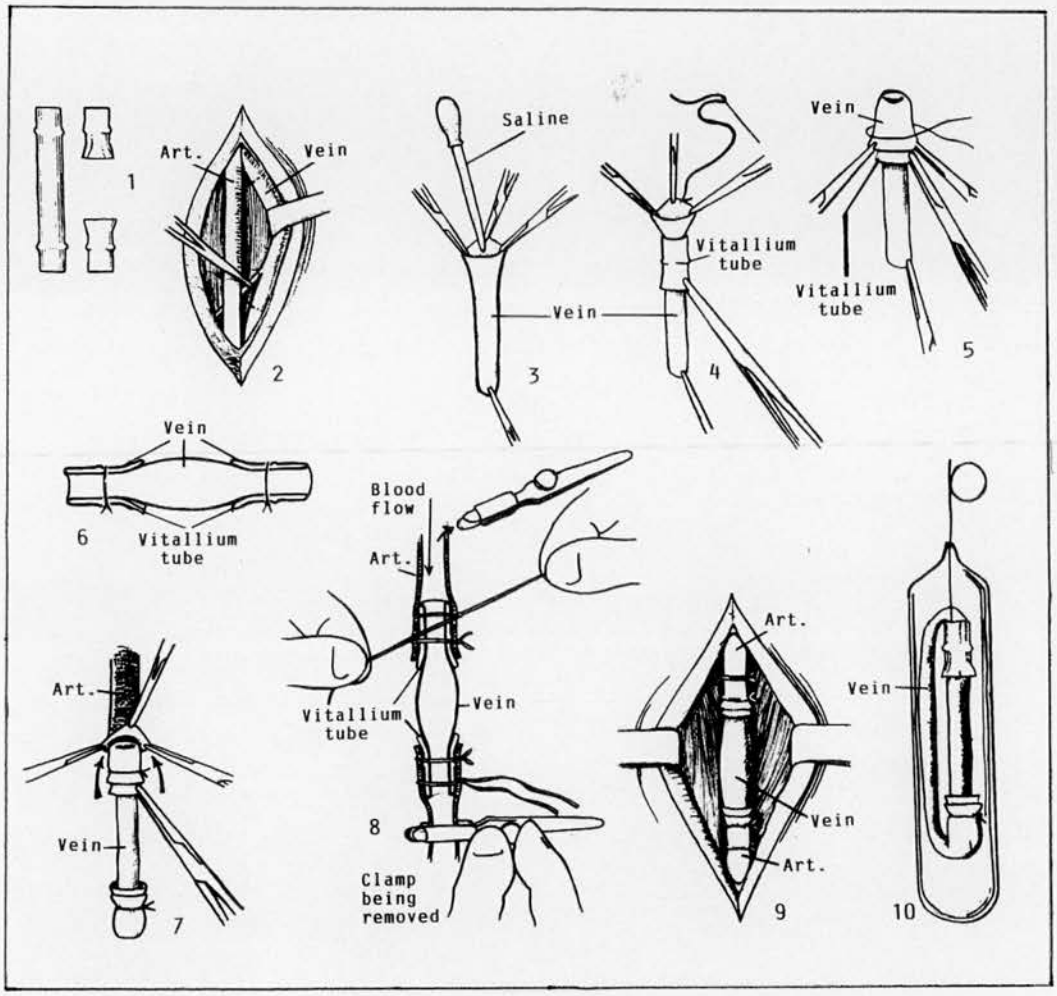


Figure 2b.

The technique used by Blakemore in the non-suture anastomosis of arteries, taken from the same source as Figure 2a.

In 1902, Alexis Carrell published his first paper on vascular anastomosis and transplantation.[34] He was the first to demonstrate that end to end arterial suture was consistently possible using his triangulation technique, and the first to use venous autografts in dogs, work for which he was awarded the Nobel Prize in 1912. From this, Carrell proceeded to use interposition vein autografts for trauma, and developed organ transplantation.[35,36]

In the years following Carrells original publication, autogenous vein grafting was used by Goyannes (1906)[37] and Pringle[38] (1913) for popliteal aneurysm, and by Lexer (1907)[39] for repair of an axillary aneurysm. Vein grafts were used in World War 1 in cases of arterial trauma in German casualties, and a large series was reported by Weglowski.[40]

Although these developments in anastomosis and autografting were to have widespread implications later in the 20th Century, they went largely un-noticed in the first two decades. Progress in aneurysm surgery was slow, and surgical procedures for peripheral aneurysms was almost entirely obliterative. Reids paper from the Johns Hopkins Hospital details the entire experience of that unit in aneurysm surgery from 1889 to 1922 as 142 cases.[41] It is interesting to note that in Reids opinion, the Antyllian operation remained the best procedure for femoral aneurysms.

Abdominal Aortic Aneurysms

Aneurysms of the body cavities remained neglected and untreatable following their initial description by Vesalius. It was not until the early 19th century that the first steps were taken to effect cure of abdominal aortic aneurysms, beginning in 1817 with another renowned experimental surgeon, Astley Paston Cooper. Following his proof that aortic ligation in the dog was compatible with survival, Cooper performed the first ligation of a human aorta for a leaking iliac aneurysm.[42] The patient, a man, was in extremis from the extent of the bleeding. Bearing in mind the absence of asepsis and anæsthesia from the surgical repertoire, the decision to attempt operation was a courageous one for both patient and surgeon. Cooper entered the abdomen through a four inch incision in the linea alba centred on the umbilicus. Through this, he encircled the patients aorta with his finger, passed a ligature, and tied it. The patient unfortunately died of shock and probable respiratory failure some 40 hours after operation.

The preserved aorta of the patient described above is shown overleaf (Figure 3).

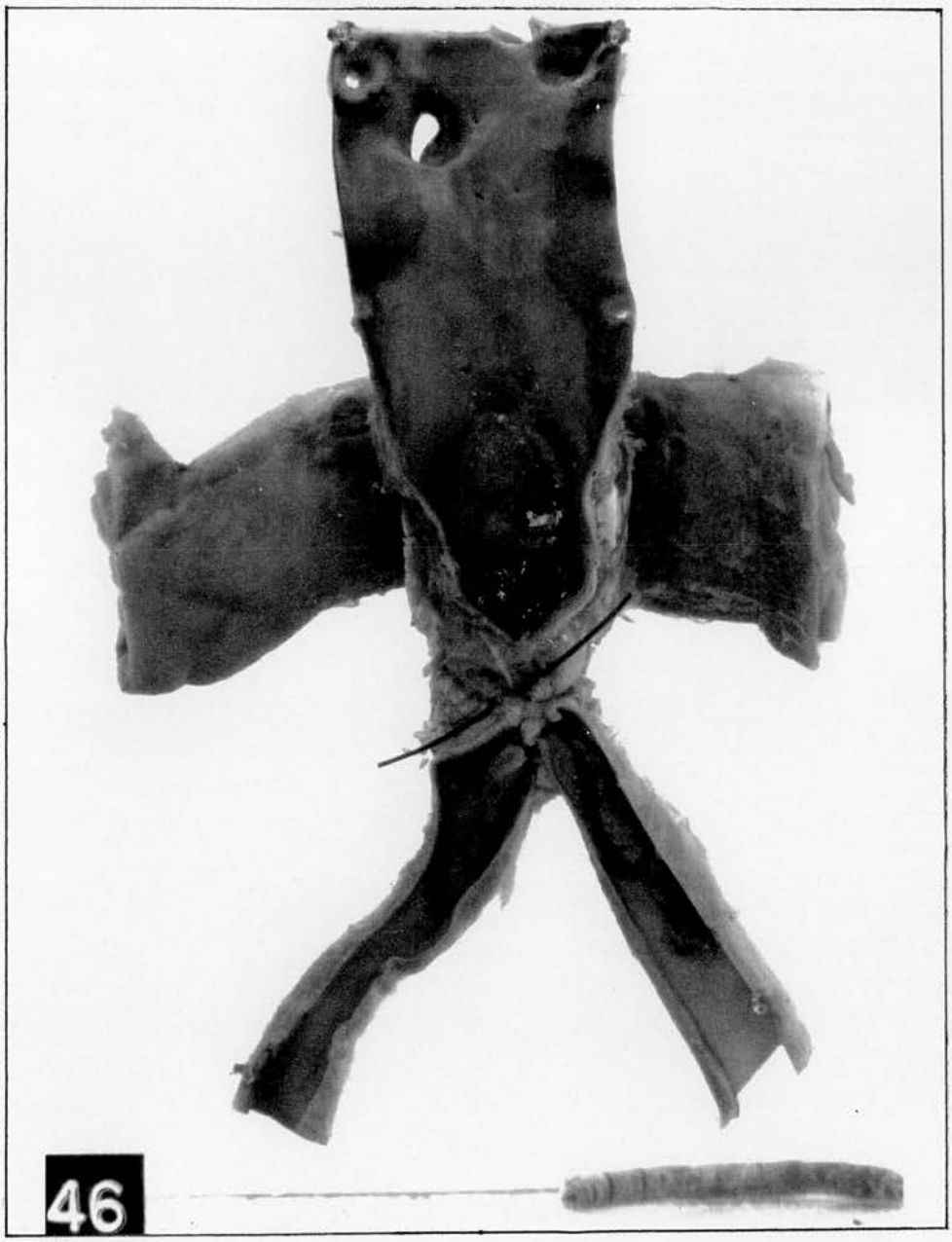


Figure 3.

The preserved aorta of the patient whose aorta Astley Cooper ligated as treatment for a leaking iliac aneurysm. The ligature can still be seen.

Specimen shown by kind permission of the United Medical & Dental Schools of Guy's and St. Thomas's Hospitals, London. The specimen still resides in the Pathology Department of St. Thomas's Hospital.

In the wake of historical experience of obliterative therapy in peripheral aneurysms, thrombosis of aortic aneurysms was attempted by a number of methods. In 1831, Velpeau attempted to produce thrombosis of an abdominal aortic aneurysm by placing three pairs of sewing needles in the sac.[43] This was followed in 1864 by Moore's introduction of 26 yards of fine iron wire into the ascending aortic aneurysm of a young man in order to produce thrombosis within the sac.[44]

Refinements of the technique of aneurysm thrombosis by wiring were produced by Matas, Hunner and Blakemore in the period up to 1947. These mainly depended on the insertion of a length of wire in the aneurysm sac, and the passage of a current through the wire to produce electro-coagulation.[45-47]

The practice of aneurysm wiring persisted until very recently, especially as an option in patients considered to be unfit for prosthetic replacement. In 1968 Peacock described a series of 14 patients in whom aneurysms were wired, with good post-operative results.[48]

An aneurysm wired by Professor J.H. Peacock, formerly Professor of Surgery at Bristol University is illustrated below (Figure 4a). The instrument used by Professor Peacock for wiring aneurysms is shown in Figure 4b.

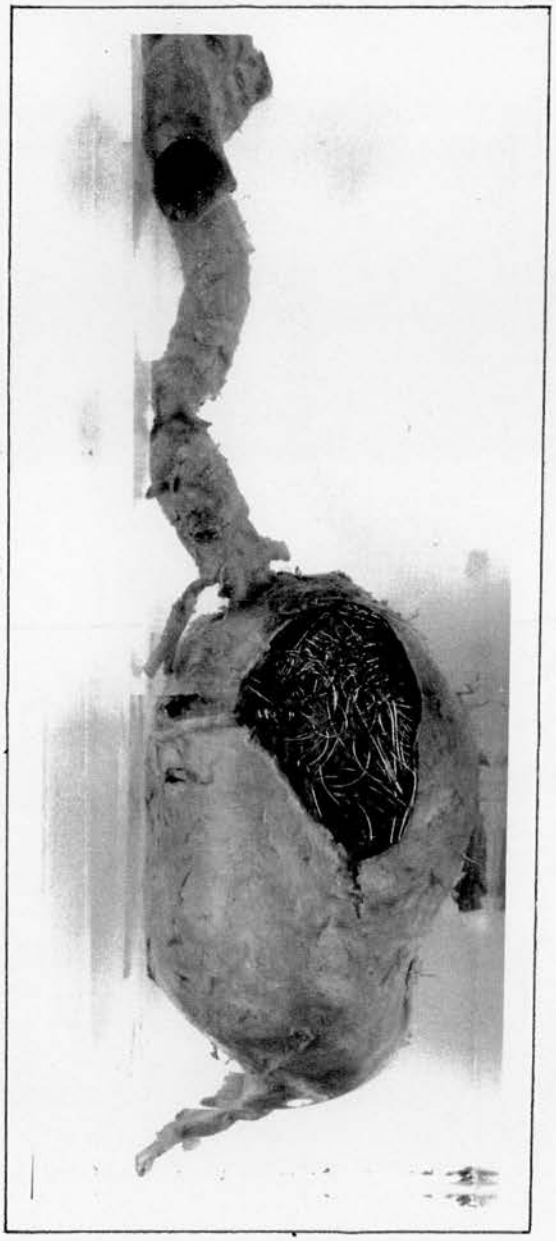


Figure 4a.

An aortic aneurysm wired by Professor J. H. Peacock of Bristol Royal Infirmary. The coils of wire can be clearly seen within the aneurysm sac.

The patient survived for more than 10 years after the wiring until he collapsed and died of rupture of the aneurysm.

Specimen shown by kind permission of the Department of Surgery, Bristol University, in whose museum the specimen resides.

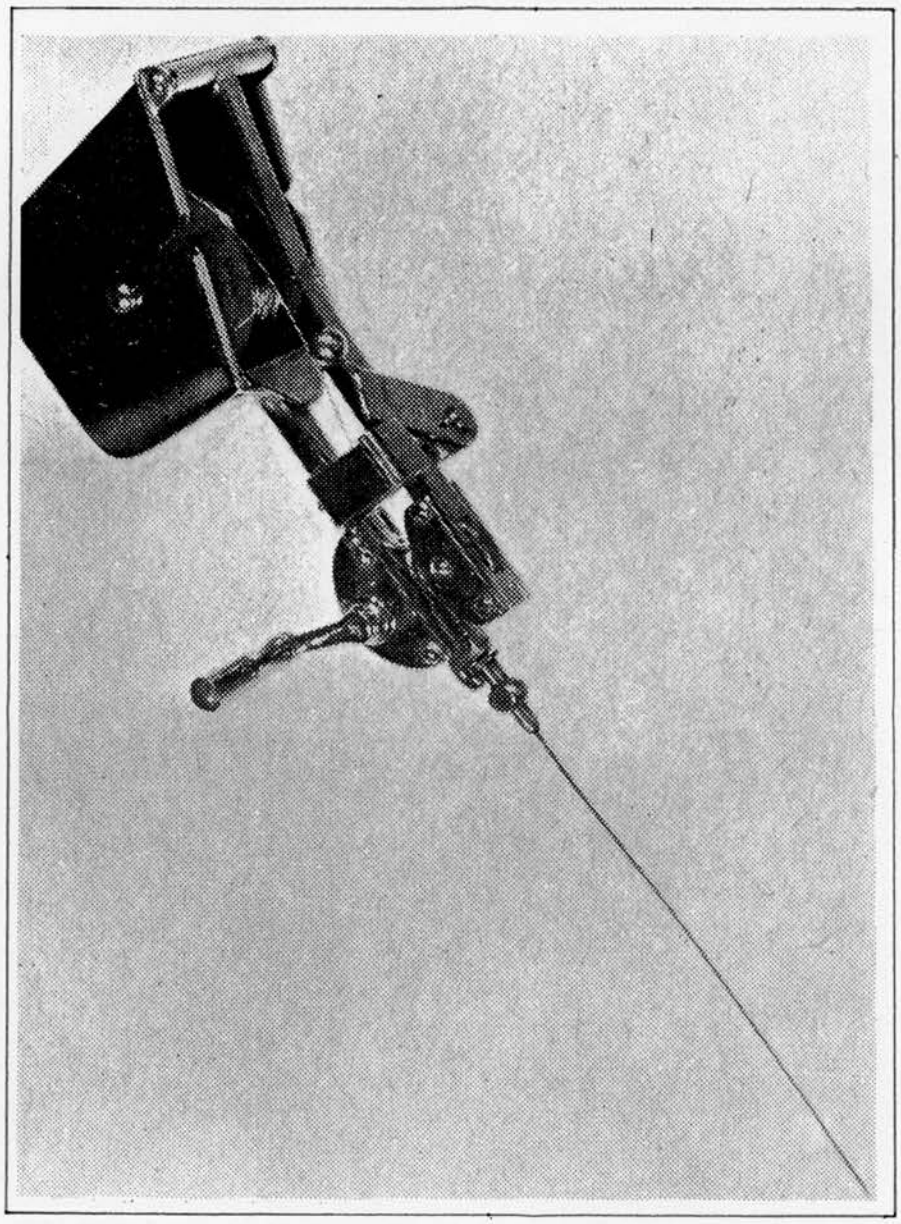


Figure 4b.

The machine used by Peacock to wire aneurysms. A spool of wire is contained in the proximal end. The wire then passes between two milled wheels, the lower of which is turned by a small handle. Counter force is applied by manual pressure on the upper wheel. By this mechanism the wire is 'friction fed' through the hollow needle into the aneurysm.

Simultaneously, attempts were continuing to successfully ligate the abdominal aorta as treatment for aneurysm. This was eventually achieved by Matas in 1923, although the ligation was found to be incomplete.[49] The first complete ligation with a satisfactory outcome was performed by Barney Brooks in 1926.[50] There followed a number of reports of aortic ligation with survival of the patient, including one restorative endaneurysmorrhaphy carried out by Bigger in 1940.[51]

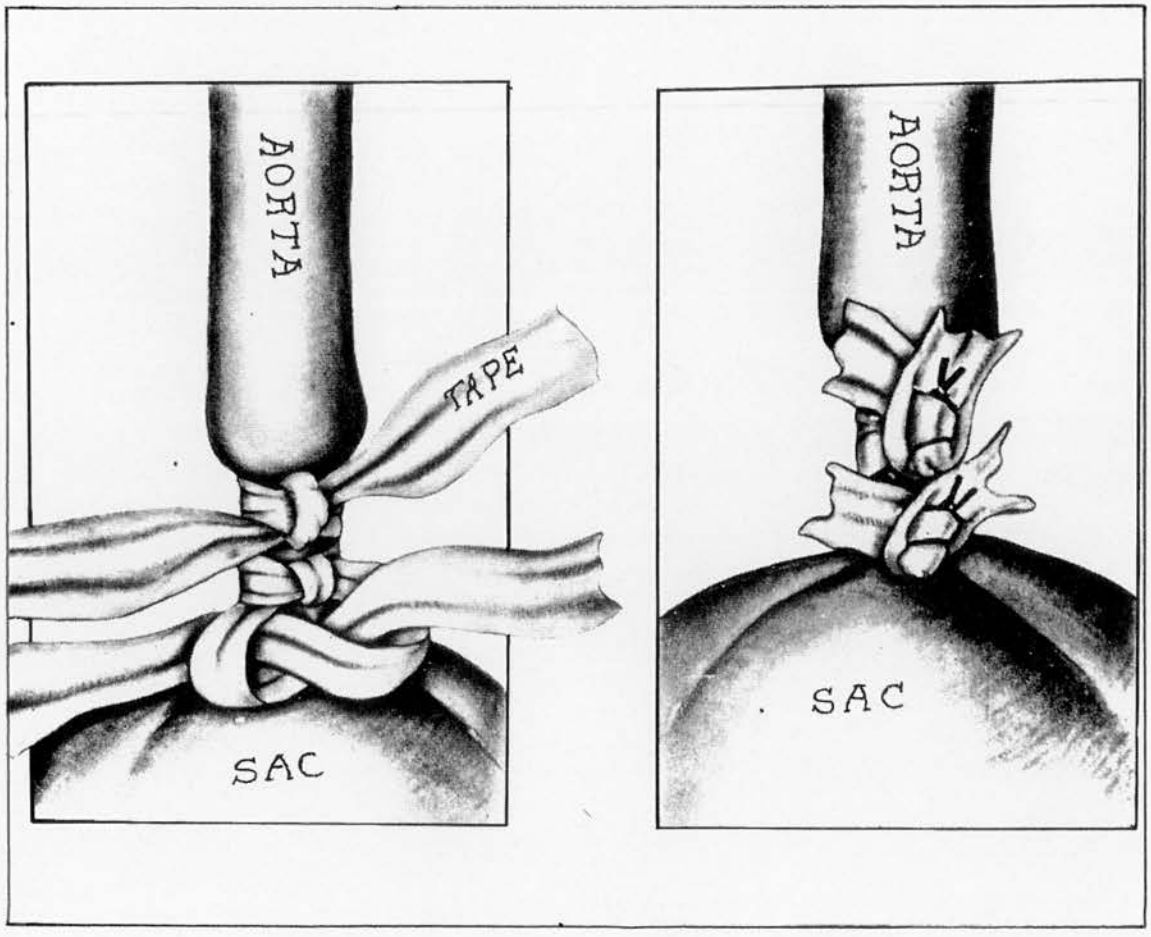


Figure 5.
The method of aortic ligation used by Matas in 1923.[49] Two linen tapes were used, tied at the aneurysm neck. The tapes were themselves transfixed with silk ligatures.

As for the treatment of peripheral aneurysms, the treatment of aortic aneurysms was limited to obliteration of the aneurysm by thrombosis, albeit by a number of different methods, until the late 1940s.

With the introduction of heparin into clinical use by Murray in the 1940s[52] came the ability of surgeons to operate during a prolonged 'safe' period. Following the description of femoral endarterectomy by J.C. dos Santos,[53] and its development by Cannon and by Bazy,[54,55] this procedure was used for the treatment of aortic occlusive disease by Freeman and Leeds in 1951[56] following the technique of Blakemore.[57] In the same year, Wylie reported a series of aortic endarterectomies for aneurysm and occlusive disease, using fascia lata wrap to reinforce the aortic reconstruction.[58]

In common with Matas' restorative endaneurysmorrhaphy,[26] Wylie's method persisted in its use of native, diseased arterial wall to restore arterial continuity.

Concurrently with the success of Wylie and others in reconstructive techniques, other groups were considering the other option - replacement of the aneurysmal segment with an alternative conduit.

During his early work, Carrell had had some success in the preservation of aortic allografts using refrigeration.[59] This work was further developed by Gross, who used preserved allograft aorta in the replacement of long aortic coarctations in 1948. He used a preservative solution of antibiotics and glucose, together with cooling between 1 and 4 degrees C., and achieved good results.[60,61] Significantly, it was Gross's preservation technique which was used for the grafts employed in the first operations for replacement of an occluded aorta (Oudot 1951) and aortic aneurysm (Dubost 1952). Work on

the preservation of aortic homografts by rapid freezing in air initially pioneered by Hufnagel[62] was continued by Deterling.[63] This led to the development of other preservation techniques,[64,65] with progression to banks of allograft arteries in the early 1950s.

The first human artery bank in the world was installed at St. Mary's Hospital, London, as a result of collaborative work between Hufnagel and Eastcott, and used a rapid deep freezing technique allowing indefinite storage.[66]

In 1951, successful replacement of an occluded aortic bifurcation was reported by Oudot using a preserved aortic allograft.[67] His patient was a 51 year old female who had claudication, rest pain and aortic occlusion. There was ulceration and tissue loss over the left lateral malleolus. Oudot adopted a retroperitoneal approach to the aorta and bifurcation, and excised the thrombosed vessel from inferior to superior, hampered by some peri-aortitis. The allograft, which had been preserved in the manner of Gross, was sutured to the aortic stump and distally to the external iliacs. Good flow was achieved down the left leg of the graft, but the right had thrombosed by the end of the operation. The patient developed some patchy blueness of the right leg, which resolved over 48 hours with heparin therapy. The left sided ulcers healed, and the patient did well.

In the face of developing enthusiasm for aortic surgery it was simply a matter of time before similar techniques were used to replace aortic aneurysms. This was accomplished successfully by Dubost on the 29th of March 1951, again using allograft aorta, following Schaffers unsuccessful attempt earlier the same month.[68,69]

Thereafter there was a rapid adoption of Dubosts techniques by Julian, Brock, and DeBakey & Cooley in Houston in the late 1950s and early 60s.[70-72] By 1953, Bahnson had reported a series of 17 patients from Johns Hopkins who had undergone replacement of aortic aneurysms. Five of the six patients who had undergone replacement of infrarenal aneurysms had survived.[73]

Initial justification for the explosion of aortic surgery in the early 1950s was not difficult. In 1950, Estes published a pivotal paper on the natural history of abdominal aortic aneurysms, showing a five year survival rate without treatment of under 20% [74]. Gleidman went on to show that the mortality for *symptomatic* aneurysms was considerably higher at 80% in 6 months; much greater than in the group of patients with asymptomatic aneurysms[75]. These findings have subsequently been confirmed by a number of other authors. Szilagyi's appraisal in 1966 emphasises the success of the surgical treatment of aneurysm in the elective situation, and confirms the earlier findings of Estes.[76] The rapid increase in aortic aneurysm surgery has decreased the ratio of patients presenting with ruptured aneurysms to patients undergoing elective surgery, and has improved the mortality figures for such elective surgery considerably.

Currently aneurysm surgery is carried out almost as a routine following diagnosis. Trends to earlier and earlier diagnosis are leading to the detailed examination of the use of ultrasound screening for aortic aneurysms among the increasingly elderly population.[77]

Once the diagnosis is established, the operative mortality for elective prosthetic replacement of an aortic aneurysm is about 5% in most centres. Following successful replacement, the patient can look forward to a lifespan virtually identical to normal individuals of the same age.[78]

Aortic Replacement - alternatives to the allograft.

Aortic allografts played a major role in early programmes for aortic replacement. Patency rates were good, at 90% in the short term,[79] but there were a number of important drawbacks to the use of allograft material. Not only was the availability of allografts limited, but preparation and storage were time consuming and expensive, although developments in rapid freezing had improved this.

Long term follow-up suggested that allograft aorta deteriorated after long periods of implantation. Carrell had suggested that the long term performance of allograft aortic replacements in dogs was good, despite the replacement of the muscle and elastic tissue of the graft wall with connective tissue.[80] A larger study, again using the dog model, was performed by Coleman and revealed a high incidence of significant thinning, calcification and loss of elastic tissue in allograft segments. There was, however, in confirmation of Carrels findings, no apparent hæmodynamic consequence of these findings of degeneration.[81] In view of equivocal reports emerging in the use of synthetic fabric prostheses, Coleman continued to recommend the use of allografts.

In the light of increasing evidence that early elective surgery was indicated in aneurysm disease of the abdominal aorta, there was pressure to find a cheaper, more readily available alternative to allograft substitutes for the aorta. Clearly the answer to most of the problems associated with the availability of allografts could be answered by the use of synthetic prosthetic grafts.

Early attempts in the use of synthetic prostheses for vascular replacement met with little success and had a prohibitive occlusion rate.[82-84] Some encouraging results were obtained by Hufnagel in 1947 using methyl

methacrylate tubes in dog aortas,[85] but a singular lack of success was reported by Moore and Donovan using polyethylene.[86,87]

Voorhees revived interest in synthetics as arterial grafts, and by 1952 had demonstrated the viability of fabric arterial substitutes made of Vinyon-N (a co-polymer of vinyl and nylon) in the aorta of experimental animals.[88]

In the wake of Voorhees work, rapid development of synthetic fabric arterial substitutes took place. A number of materials were tried, including Nylon, Terylene, Orlon, Ivalon, Teflon and Dacron in a variety of sites, but most were found to be unsatisfactory.[89,90]

Two reports reflect the active search for acceptable vascular prostheses, and reach conclusions based on sufficient numbers.[79,91] Both of these major studies found that, in view of the long term loss of tensile strength that occur with Orlon and Nylon, and the formidable thrombogenicity of Ivalon, that Dacron and Teflon were clearly the best of the available materials.

Since that time, basic materials used in arterial surgery have changed little, most products being a slight modification on the original P.T.F.E. and Dacron.

Thoraco-abdominal aneurysms

In 1955, Ellis demonstrated that repair of the aorta was possible when the renal vessels were involved, and that renal function could be preserved.[92] The patient, a 64 year old male, presented with a symptomatic aortic aneurysm. The level of the left renal artery was normal, but the right arose well below the inferior mesenteric artery. The right renal artery was clamped, then re-anastomosed to the aortic allograft following its insertion.

The total renal ischæmic time was 135 minutes. The patient made a full recovery with arteriographic and urographic evidence of continued renal artery patency and renal function.

In the same year, Etheridge undertook the replacement of a large thoraco-abdominal aneurysm with re-implantation of the coeliac and superior mesenteric vessels.[93] Etheridge's patient was a 37 year old construction worker. At operation, the aneurysm was found to include the origins of the superior mesenteric and coeliac vessels, and to involve the left kidney in a mass, requiring resection of the kidney with the aneurysm. The inferior limit of the aneurysm lay above the level of the right renal artery, and a polythene shunt was used to bypass the aneurysm from the thoracic aorta to the bifurcation. Thus the right kidney was perfused throughout the operation. Post-operatively the patient did well apart from a successfully managed episode of acute renal failure, and was back at work and well a year later.

The anatomy of the aneurysm described above, and the techniques used in its repair are illustrated in Figures 6a-6c.

Figure 6a.

The anatomy of the aneurysm repaired by Etheridge in 1955.The aneurysm was densely adherent to the left kidney, and involved the origin of the coeliac and superior mesenteric arteries.

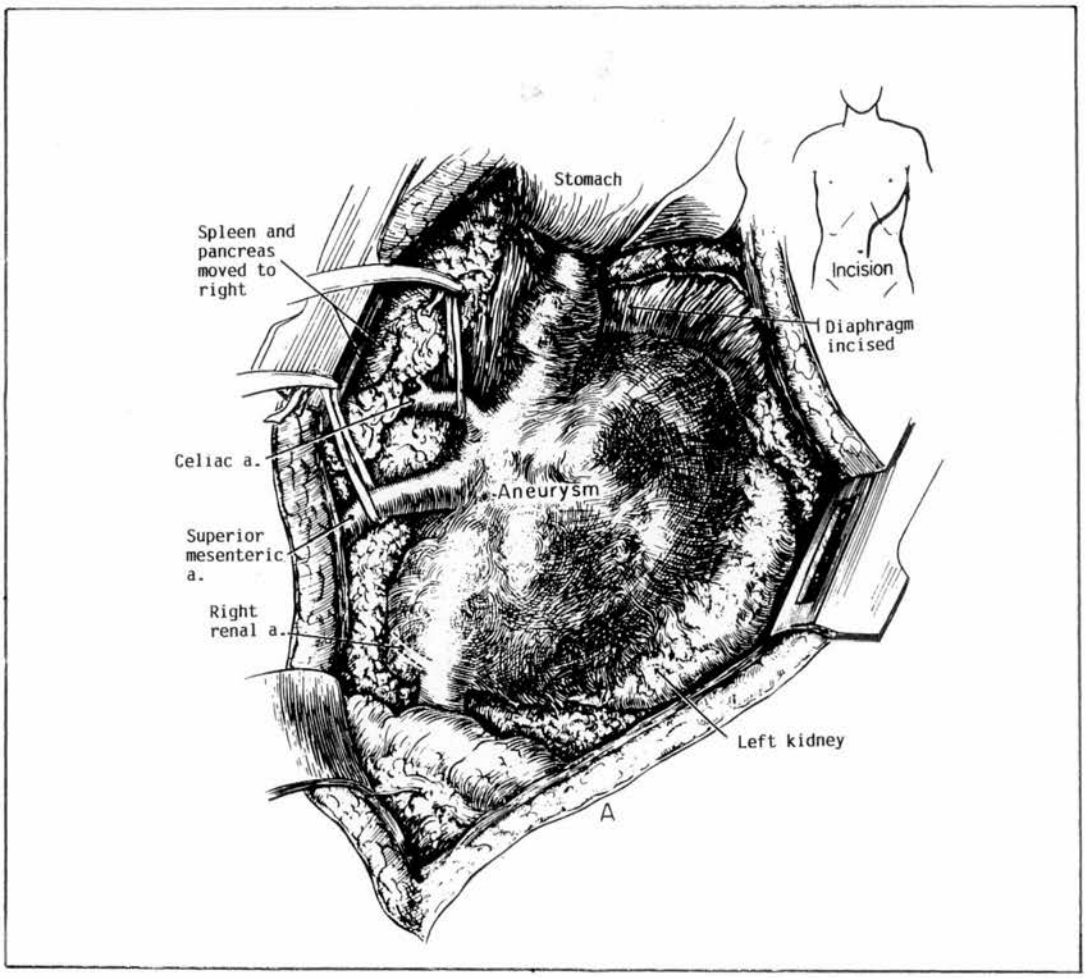


Figure 6b.

The technique which Etheridge used to repair the aneurysm. The polythene shunt can be seen sutured in place, and the aorta has been divided proximal to the neck of the aneurysm.

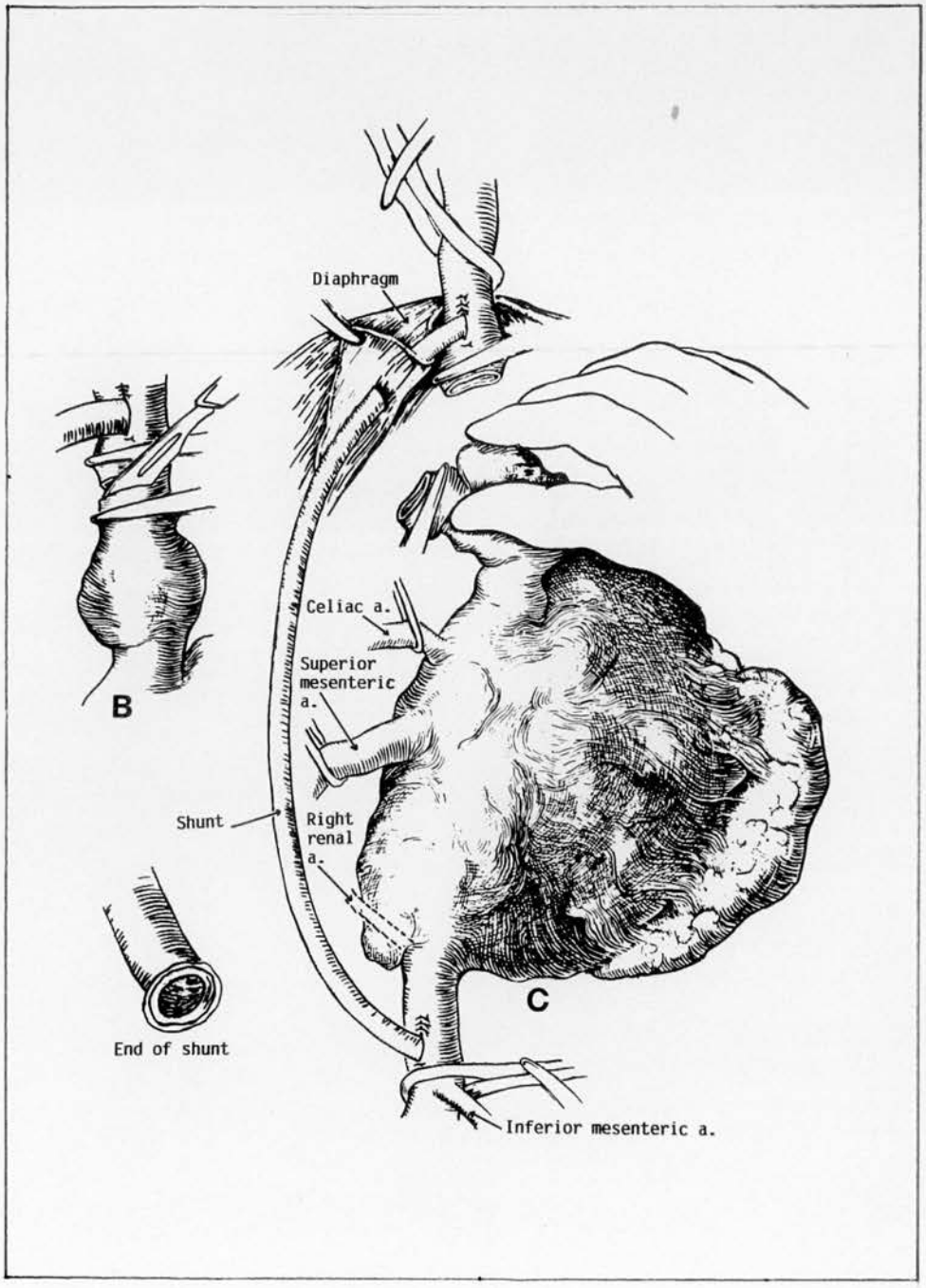
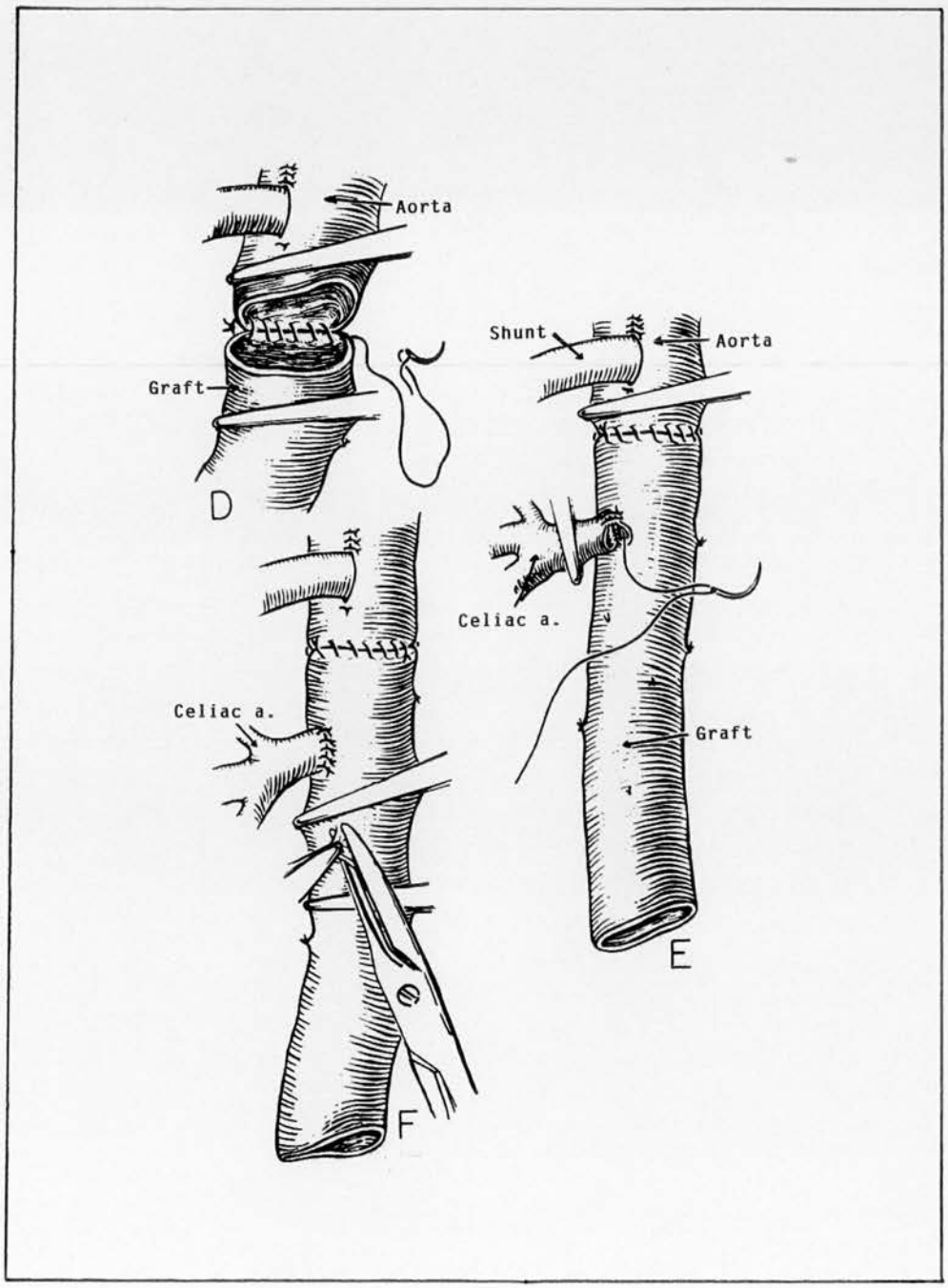


Figure 6c.

A detail of the operative technique used by Etheridge, showing completed anastomosis of the coeliac artery to the graft. An opening is being made for anastomosis of the superior mesenteric artery.



This was followed by a number of papers describing methods for the re-anastomosis of visceral branches. The culmination of the technical approach to thoraco-abdominal aneurysms was achieved by Stanley Crawford , with the publication of his paper 'Thoraco-abdominal and abdominal aortic aneurysms involving renal, superior mesenteric and coeliac arteries' in 1974. The classification of thoraco-abdominal aneurysms, and the operative techniques pioneered by Crawford have not been bettered. Both are still in routine use.[94,95]

Figure 7a & b show Crawfords technique for the repair of a ruptured thoraco-abdominal aneurysm in a patient who had previously undergone repair of an abdominal aortic aneurysm. The illustrations are reproduced from the original paper.[94]

Figure 7a.

In E and F. clamps have been applied proximal and distal to the aneurysm, and the aneurysm opened longitudinally. The edges of the sac are retracted to display the orifices of the major visceral arteries.

G-I show the proximal aortic anastomosis being made, and the coeliac artery anastomosis to a circular opening in the graft.

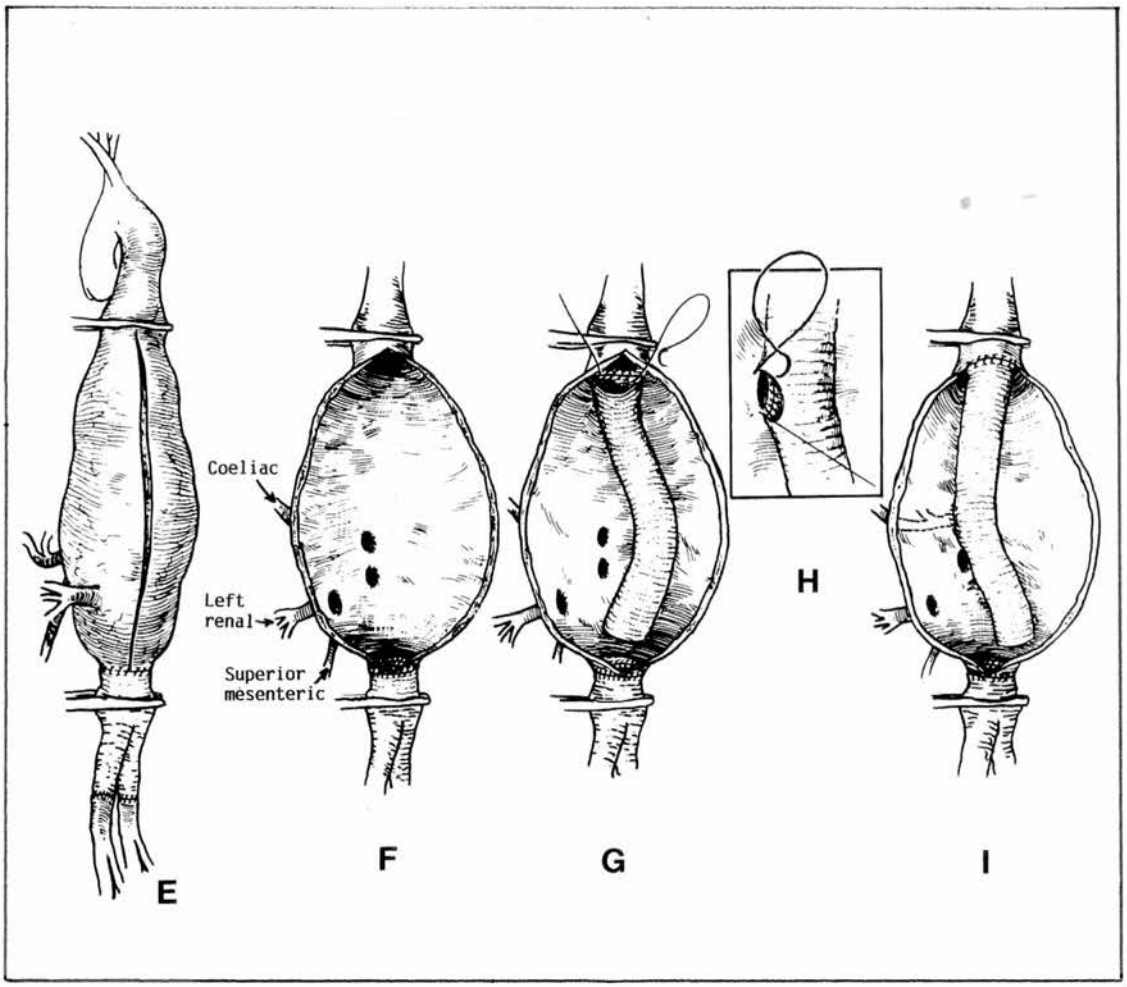
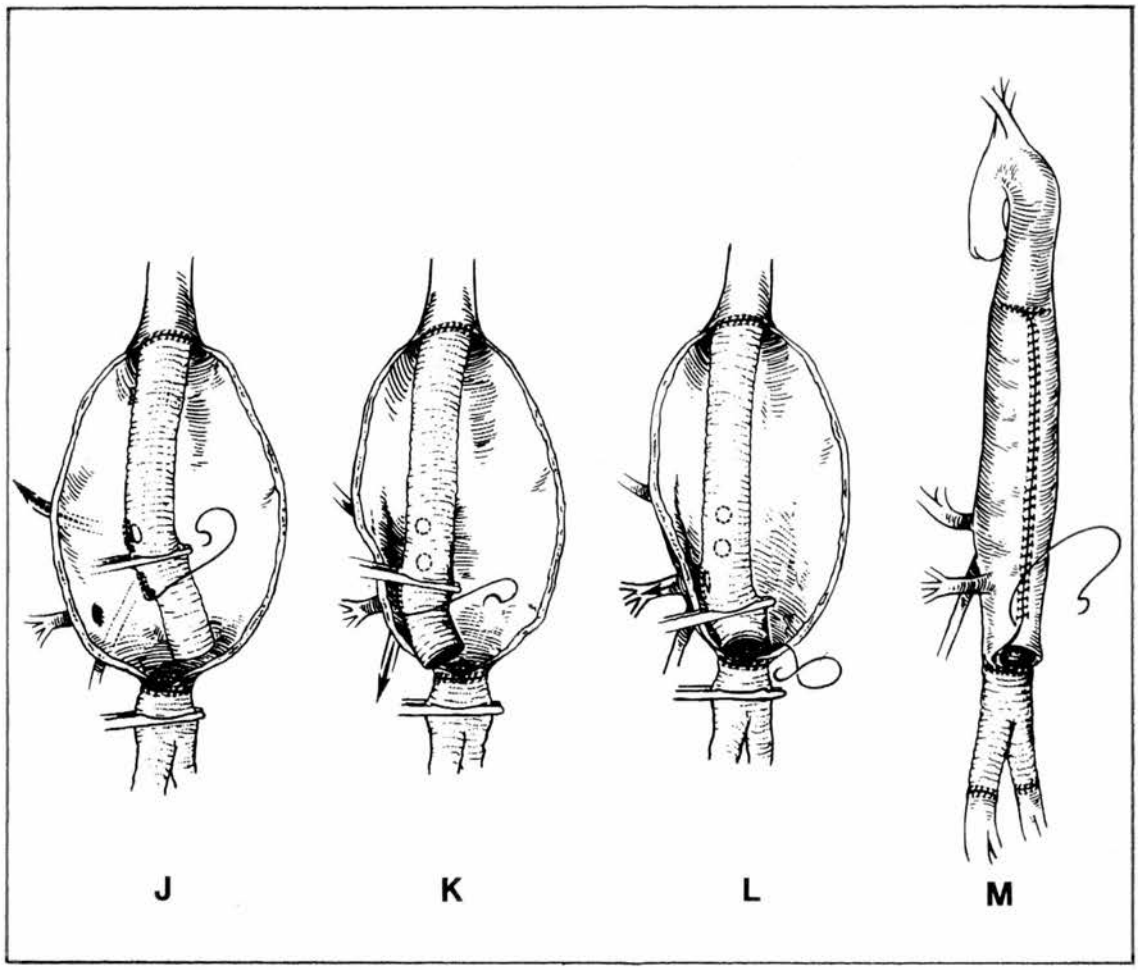


Figure 7b.

J & K illustrate the serial completion of anastomoses between major visceral arteries and the aortic graft. The lower aortic anastomosis and closure of the aneurysm sac are shown in L & M.



Inflammatory Aneurysms

What is probably the first recorded case of inflammatory aneurysm was published in the British Journal of Urology by T.G.I. James in 1935,[96] (See Appendix 1). He documented a case of uræmia in a female patient, who was also found on examination to have an aortic aneurysm. At post-mortem examination, the aneurysm was found to be thick walled and to extend from the bifurcation up to the level of the superior mesenteric artery. The renal arteries were encased in the aortic wall and the ureters densely adherent. The size of both kidneys was reduced. The Wasserman reaction had been noted to be negative.

In 1955, De Weerd et al.[97] undertook the first operative treatment of ureteric obstruction by inflammatory aneurysm fibrosis. Bilateral ureterolysis was performed, but the aneurysm not resected. The patient did well, with resolution of the uræmia. Later that year, Shumaker and Garrett[98] performed the first resection of an inflammatory aneurysm. Their 60 year old male patient presented with pulmonary oedema and uræmia in the presence of a 10 cm. aortic aneurysm. Fibrotic tissue surrounded the aneurysm, and involved the sigmoid colon, jejunum, and both ureters. Prosthetic replacement of the aneurysm was carried out with a fused nylon/polyethylene Y graft, and bilateral ureterolysis was performed.

Over the next few years several case reports and small retrospective series of abdominal aortic aneurysm in association with severe fibrosis and ureteric obstruction appeared in the literature.[99-101] It was only following Walkers detailed review of the clinical features and pathology of 19 cases in 1972 that the term 'inflammatory aneurysm' was coined, and the disease formally recognised.[102]

Despite the emergence of inflammatory aneurysm as a distinct clinical entity little interest was aroused in its clinical or pathological features. Owing to the rarity of the condition, even vascular surgeons encountered inflammatory aneurysm only moderately infrequently. This is reflected at least in part in Goldstones[103] inability to find any specific referral to inflammatory aneurysm in the American literature prior to 1977, save a brief mention by Wylie in a textbook in 1975.[104] The pattern of small retrospective reviews and literature reviews has persisted, highlighting the difficulties of detailed study.

Goldstone[103] in 1977 reviewed a smaller series of inflammatory aneurysms than did Walker (10 patients as opposed to Walker's 19), but went some way further to pointing out the dangers of operating on such aneurysms. He also suggested some modifications to operative technique which might be used to surmount these problems. In particular, he suggested the minimal dissection technique which is still standard practice today.

The topic of inflammatory aneurysms was briefly reviewed by Olcott in 1978, in a paper outlining unusual clinical manifestations of abdominal aortic aneurysms.[105] In 1981, Rose & Dent first proposed that inflammatory aneurysms may simply represent an extreme manifestation of atherosclerotic aortic aneurysm disease, in their review of the pathological features of 51 aortic aneurysms including six of the inflammatory type.[106] Theirs was the first attempt to produce a histopathological study comparing inflammatory and non-inflammatory aneurysms on the basis of a pathological grading system. Their review, although detailed, was descriptive only, and made no firm commitment to the possible ætiology of inflammatory aneurysm. More recently a number of authors have proposed ætiological mechanisms for inflammatory aneurysms, and these are dealt with in detail in chapter 3.

DEVELOPMENT OF AORTIC IMAGING TECHNIQUES

The practice of surgery has been revolutionised in the past 20 years by advances in radiological techniques, and now the optimisation of vascular surgical treatment is heavily reliant on the results of radiological investigations.

Plain Radiography

Plain radiographs are of limited use in the diagnosis of aneurysm of the aorta. The lumen cannot be imaged, and the walls of the vessel can only be seen if they contain calcified atheroma. Other cheap and easily available methods are available which provide more information in cases of suspected abdominal aortic aneurysm. In this circumstance, and for follow-up assessment of aneurysms, plain radiology may rightly be viewed as an un-necessary radiation exposure. Aneurysmal dilation of a calcified aorta may be seen incidentally on plain radiographs taken for other suspected pathology, and prompt clinicians to investigate further, but this is the only 'use' of plain films. For this reason, plain radiography is not discussed further in this or succeeding chapters.

Aortography

Following Roentgens discovery of x-rays in 1895, it was a matter of a few weeks until Haschek and Lindenthal performed arteriography in a cadaver using chalk as a contrast medium.[107] There followed a delay of 34 years before arteriography was performed in a live human subject by Dos Santos et al., who used direct intra-aortic injection of sodium iodide to produce

the first aortogram.[108] A similar technique was used by Nuvoli in 1941, who chose the ascending aorta as his injection site.[109]

In 1941, Farinas used a percutaneous catheter in a human subject to carry out transfemoral aortography [110], but it was not until Seldingers introduction in 1953 of the technique which still bears his name, that arteriography advanced substantially.[111]

The basis of the Seldinger technique is the introduction of a guide wire into the arterial lumen through a small needle. A catheter is then passed over the wire and into the artery. In this way, the catheter can be of greater diameter than the needle, and the wire can be used to assist and direct its passage in the arterial lumen. It is this technique which has become the cornerstone of modern arteriography.

Since the use of organic iodide in 1929 by Swick, a number of intra-arterial contrast media have become available.[112] Most of the immediate successors of Swicks organic iodide were variations on that theme, producing allergy and a variety of neurological, renal and cardiopulmonary toxic effects. In the early 1970's, Almen developed metrizamide, a non-ionic contrast medium of low toxicity.[113] Newer agents, such as iohexol, of the same general characteristics have since become available, and are cheaper and easier to handle.

The development of digital subtraction angiography (DSA) in the late 1970's has changed the use of aortography in aneurysm disease. The technique uses fluoroscopy to collect intensified images. The image information is subsequently processed to subtract extraneous details such as bone, and to enhance contrast. Although the dose of contrast medium needed is considerably reduced in DSA, images still may suffer from motion artifact, and from a lack of spatial resolution for this reason.[114,115]

Aortography is an invasive technique, and has complications related to the delivery of contrast into the arterial tree, some of which are due to the contrast medium, and some to arterial catheterisation. Adequate hydration and the use of non-ionic contrast media have limited the incidence of renal failure due to contrast media, as has the increasing use of DSA, with the resulting use of small doses of contrast. Allergic, neurological and cardiopulmonary complications continue to occur, but their incidence is extremely small.[116,117]

Catheter related problems include haemmorhage, pseudoaneurysm formation, thrombosis, arteriovenous fistula formation and embolisation.[118] The complication rates are approximately 0.5% for transfemoral catheterisation, 0.6% for the translumbar route, and 2% for transaxillary catheterisation.[117]

Ultrasound

Originally derived from sonar, (**so**und **na**vigation and **r**anging),[119] ultrasound was first used to demonstrate aortic diameter in 1961 by Donald and Brown using a technique similar to present day M-mode ultrasound.[120] Since then, the development of B-mode image production, and the advent of grey-scale ultrasonography have increased image definition. Ultrasound can easily differentiate aortic aneurysm from aneurysm of the visceral arteries, and can measure aortic diameter with accuracy in clinical practice.[121] The combination of B-mode and pulsed Doppler velocimetry known as Duplex scanning is now being used in the evaluation of the aorta, its major branches, and the arteries of the lower limb.[122]

Because of the wide availability and increasing improvement of machines, ultrasound has become the investigation of first choice in suspected aneurysm of the abdominal aorta. Frequently however, distortion and cephaloid displacement of the aneurysm sac, technical limitations due to probe shape and size, sound transmission through bowel gas and patients' obesity may limit the information available from ultrasound. Improvements in this area may be made in the near future when colour coded ultrasound imaging becomes more widely available.

Perhaps the most exciting use of ultrasound in the diagnosis of aneurysm of the aorta is still under scrutiny. Over the past five years increasing use of portable ultrasound machines has been made in community screening for aortic aneurysm disease. Major advances have been made in the selection of 'at-risk' populations, and studies of cost-effectiveness have suggested the advantages of screening.[123,124]

Computerised Tomography

In 1968, Godfrey Hounsfield suggested that computers and mathematics could be used to reconstruct images from the integration of sets of x-ray measurements taken from different angles. Development was so rapid, that by 1972 a computerised tomography scanner was available to image the central nervous system, and its clinical potential being explored. There followed a whole body instrument in 1975 with satisfactory resolution, and Hounsfield was awarded the Nobel prize in 1979. Since then, advances in computer technology have led to increased resolution, and a reduction in the time taken to produce the images.

C.T. uses circumferentially acquired radiographic data to reconstruct a cross-sectional image. The C.T. image represents a cross-sectional tissue slice of selected thickness from 2 mm. to 1 cm. Thinner 'slices', produce greater spatial resolution and fewer artefacts. Infusion of contrast material is necessary for adequate imaging of hollow organs such as the ureters and blood vessels. Highly accurate imaging of the renal arteries is possible, but multiple doses of contrast medium and the acquisition of many closely spaced C.T. 'slices' in the appropriate region are required. This increases the radiation dose sustained by the patient, and the time taken for scanning.

Computerised tomography is a safe procedure. Complications are related to intravenous contrast administration, and are very rare. The radiation dose sustained by patients is less than that for a barium enema in normal circumstances.

Magnetic Resonance Imaging. (MRI)

One of the newest radiological techniques, magnetic resonance imaging was first used as an analytical tool in chemistry in the 1949s.[125] The localisation of resonating nuclei in one dimension was reported by Gabillard in 1952,[126] and in 2 dimensions 20 years later by Lauterbur.[127] The first use of M.R.I. in human imaging studies was by Hinshaw et al. in 1977, when they produced a cross-sectional image of a human wrist.[128] The earliest reference to the use of M.R.I. in vascular disease was in 1983 by Higgins et al.[129]

The assessment of M.R.I. as an imaging technique in aortic aneurysm disease, and particularly inflammatory aneurysms forms the basis of part of this thesis.

CHAPTER 2

THE ÆTIOLOGY OF AORTIC ANEURYSMS: CURRENT THEORIES.

INTRODUCTION

The question of the ætiology of aortic aneurysms has been a continuing source of interest to both vascular surgeons and pathologists. Atherosclerosis is still thought by pathologists to be the most likely cause, but this has been recently challenged by a number of authors.

The factors precipitating abnormal dilation of the abdominal aorta may be considered under a number of categories.

1. Mechanical factors leading to structural weakening of the aortic wall, or producing abnormal flow or pressure patterns within the aortic lumen. This includes the effects of atherosclerosis in thinning the aortic media or narrowing the aortic lumen.
2. Abnormalities of the blood supply of the aortic wall.
3. Metabolic aberrations. This includes factors leading to flaws in the structural proteins of the aortic wall in quantity or at the molecular level. Manufacture of abnormal elastin or collagen, or increased proteolysis of either protein should be considered.
4. Genetic associations.

The true ætiology of aortic aneurysms is unknown. It is likely, however, that more than one factor is involved, making clarification of the cause highly complex.

The theories of aneurysm formation which have been investigated and which form the basis of current speculation about aneurysm formation in general are discussed under the four headings listed above.

The specific pathological and clinical features of inflammatory aortic aneurysms will be dealt with in detail in chapter 3, and are not included in this review.

1. Mechanical Factors

The systolic surge of blood delivered to the aorta is met in young individuals by an elastic dilation and recoil of the aorta, a mechanism which effectively smooths the pulsatile cardiac output for delivery to the major viscera.[130] Changes may occur in the aortic diameter, wall thickness, and elasticity which reduce this capability and may predispose to the formation of aneurysmal dilation. These changes must be seen in the context of the normal dynamic function of the aorta which is an elastic tube subjected to pulsatile flow.

In the virtual absence now in Britain of syphilitic aortitis and mycotic aneurysms of the aorta, aortic aneurysms are most frequently associated with age and atheroma. Both of these may change the mechanical characteristics of the aortic wall either singly or in combination.

Age

Ageing arteries dilate, stiffen and develop thicker walls.[130-132] These changes are due to an age related reduction in the percentage of collagen and elastin in aortic wall, and an increase in the collagen - elastin ratio. Calcification of elastin fibres also contributes to the decrease in compliance of the aortic wall.

There is no doubt that risk factors for stenosing atheromatous disease and aneurysmal disease are largely the same. There are minor differences, such as the increased prevalence of diabetes in stenosing disease as opposed to aneurysmal disease, and the increased prevalence of hypertension and smoking in patients with aneurysmal disease compared with occlusive disease.[133] It is therefore not possible to distinguish between patients with aneurysmal disease and occlusive disease on the basis of risk factors. Study of the pathology of end stage occlusive and aneurysmal disease gives little information on the differences in ætiology or pathogenesis.

Three possibilities exist in aneurysm disease, linking atheromatous change and aneurysm formation :

1. That atheromatous change is a common root for the development of occlusive disease and aneurysmal disease, and that patients in each group differ in some fundamental way in their response to the initial lesion. Some patients progress to aneurysm formation, some to occlusive non-aneurysmal disease.
The aneurysm is *caused* by the atheroma.

2. That the co-existence of atheromatous change and aneurysmal dilation is seen because of the secondary development of atheroma in an artery already dilated by some other mechanism. The atheroma is *caused* by the dilation.

3. That atheroma and aneurysmal dilation develop by completely separate mechanisms, and one does not *cause* the other.

Animal studies provide the best available evidence that atheromatous change leads to aneurysm formation. Studies of primates, dogs and fowl all suggest that the development of atheromatous plaques predates the development of aneurysm. [134-136]

Initial changes in aortic diameter caused by atheroma may produce favourable conditions for continued aneurysmal dilation, and it is in this case in which hæmodynamic factors may be crucial.

The role of reflected pulse waves in the development of aortic aneurysm was first proposed by Blakemore and Voorhees in 1954,[137] and was later taken up by a number of other authors. The terminal portion of the abdominal aorta is unique in its proximity to the bifurcation. Such a region does not exist in any other part of the vessel. Blakemore & Voorhees suggested that two characteristics of the abdominal aorta predisposed to aneurysm formation. The first was that the aorta is tethered at both ends - at the diaphragm and at the common iliac arteries. Atheromatous change produces gradual stiffening, elongation and kinking of the aorta. This in turn results in a 'water hammer' pulse wave in the aorta, with abnormally high systolic pulse pressures. This abnormal pressure wave is transmitted to kinked, and therefore partially obstructed iliac arteries, producing a reflected pulse wave and increased wall stresses in the lower aorta predisposing to aneurysm formation.

Practical confirmation of this mechanism was provided between 1971 and 1973 by Newman and Gosling in a series of papers.[138-140]

Gosling[138] calculated that for minimal pulse wave reflection at a bifurcation, the area ratio (the sum of the cross sectional areas of the branches divided by the area of the parent vessel) should be 1.15. In this case, the bifurcation can be said to be 'matched'. They also showed that with age, the aorto-iliac bifurcation of man becomes progressively 'unmatched' with a decrease in the area ratio, and a corresponding increase in the magnitude of the reflected pulse wave. The aorto-iliac bifurcation in man is only 'matched' in infancy, when the ratio is 1.11, and reaches a ratio of 0.75 at age 50.

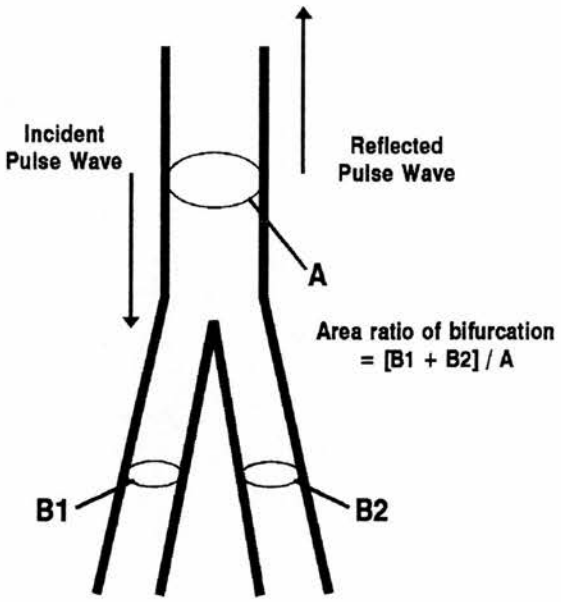
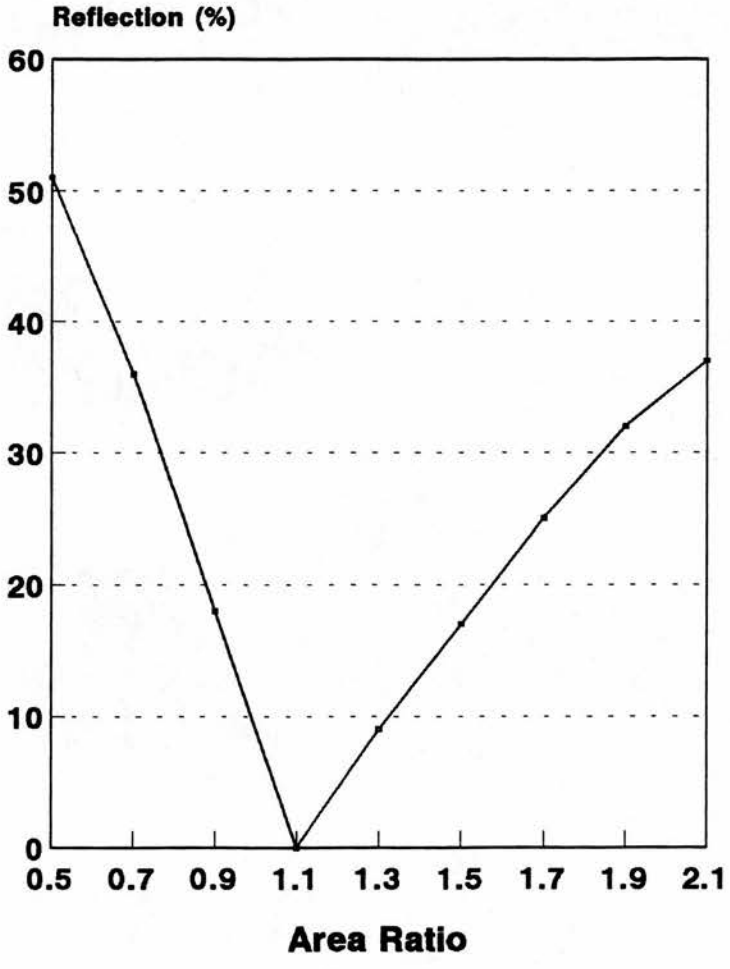


Figure 8a.

This illustrates the principle of Area Ratio. The magnitude of the reflected pulse wave varies with the area ratio.

Figure 8b.

The relationship between area ratio and magnitude of the reflected pulse wave at a bifurcation.



Newman[139] has shown in the fowl model that an increase in distensibility of the cockerel aorta occurs in the early stages of atheromatous change. This is associated with an increase in aortic diameter, and consequent mismatching of the aortic bifurcation.

Two years later, he went on to show that in direct pressure measurements in dogs with mismatched aortic bifurcations, the magnitude of the reflected pulse wave increased with decreasing area ratio.[140] An excellent distillation of this work is made in the paper from Lallemand.[141]

2. Abnormalities of Blood Supply

Structural features have been identified in the wall of the abdominal aorta which may not only explain the propensity of the infrarenal aorta to dilate, but also why it should do so more commonly than the thoracic aorta.

The formation of aneurysmal dilation of the aorta implies a localised inability of the artery wall to withstand the tensile stress imposed by the pulsatile column of blood. This inability may result from disturbance in the architecture or composition of the wall, or interference with its blood supply.

In 1967, Benjamin and Becker compared the number of vasa vasora in the thoracic and abdominal aorta.[142] They found that these vessels were abundant in the thoracic aorta, but that there was a relative paucity in the infrarenal abdominal aorta. They suggested that, following occlusion of one of the vasa vasora of the thoracic aorta by atheroma, the medial blood supply was maintained by collaterals, whereas in the abdominal aorta, such an occlusion led to medial necrosis and a tendency to aneurysm formation.

Wolinsky & Glagov and Zatina arrived at similar conclusions when they examined the medial lamellar structure of the aorta of several species of mammals and man.[143,144]

The mammalian aortic media is made up of an orderly and integrated system of fibromuscular lamellæ. Regardless of species, the number of lamellæ is proportional to the aortic diameter, such that each lamella sustains a tension of approximately 2000 dynes per square centimeter. Nutrition of the aortic wall by diffusion from the vessel lumen appeared to be sufficient to sustain only the inner 29 lamellæ. The aortæ of mammals with more than this number of aortic lamellæ possessed vasa vasora to nourish the outer layers.

The appearances of the elastic lamellæ described by Wolinsky and Glagov are shown below in Figure 9.

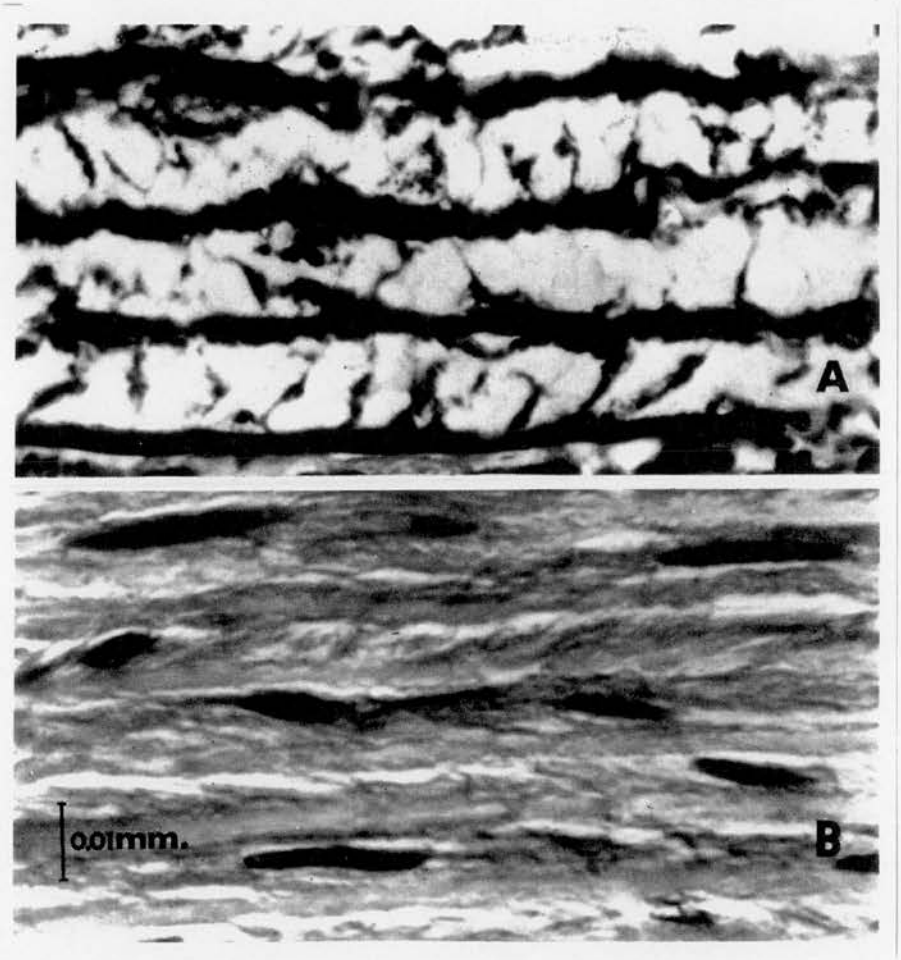


Figure 9.

Microscopic appearance of the media of the adult mamalian thoracic aorta after fixation during distention by physiological intraluminal pressure. Lamellar units consist of relatively straight elastic bands and intervening fine elastin fibres collagen fibres and smooth muscle cells. A is stained by the Weigert-Van Gieson method, and elastic fibres appear black. In B, the wall is stained with H & E. Cell nuclei appear black, and elastic fibres appear white.

Photograph from original paper by Wolinsky & Glagov.[143]

The thoracic aorta of man is furnished with a number of lamellæ and vasa vasora commensurate with its diameter, and is comparable with other mammalian morphology. In marked contrast however, the human abdominal aorta contains only 28 lamellæ, when, from animal studies, it should contain many more. This results in each lamella sustaining an abnormally high tension, of approximately 3000 dynes per square centimeter. In addition, in confirmation of the work of Benjamin & Becker, there is little evidence of vasa vasora. The net result is that in the human abdominal aorta there exists an unusual situation in that each aortic lamella carries an abnormally high load in the virtual absence of vasa vasora. This would suggest why the human abdominal aorta is particularly susceptible to aneurysm formation.

3. Metabolic Abnormalities

The tensile strength of the aortic wall is due to the presence not only of smooth muscle fibres, but also of considerable quantities of the structural proteins collagen and elastin. A decrease in the quantity, structural or functional integrity of any of these components may lead to weakening of the aortic wall and a tendency to aneurysm formation. In order to explain the changes in the aortic wall leading to aneurysm formation, which may be due to changes in elastin or collagen, a brief review of the contribution of each to normal vessel mechanics is required.

In normal vessels, at physiological pressures, arterial walls undergo distension and recoil in response to the arterial pressure wave. In this situation, the arterial wall is extremely compliant, a property due to the wall content of elastin. Elastin fibres can be stretched to 50-70% of their resting length, acting like folded-up chains. As the fibres are loaded, length increases as the chains become unfolded. As the fibres reach the limit of their ability to stretch, they become progressively stiffer, limiting further extension. Collagen, the other major structural protein, has a fundamentally different structure. Because of extensive cross-linking, collagen has a maximum extensibility of only 2-4% of resting length.

In physiological conditions, elastic and collagen fibres are stretched but bear load to different degrees. Cox has estimated that, in canine arteries under normal conditions, only 8-25% of the collagen is load bearing.[145]

With increasing age and hypertension, human arteries become stiffer. This correlates with an increased ratio of collagen to elastin in the wall. This change in connective tissue content does not account entirely for the change in stiffness. As arteries dilate with age, elastin fibres reach their extension limit, and become stiffer. At the same time, there is recruitment of collagen fibres, which are inherently stiffer, in both the media and the adventitia. In addition, there is increasing calcification of elastic fibres, further raising stiffness.

The effects of collagen and elastin destruction have been extensively studied by Philip Dobrin.[146] In essence, he has studied the behaviour of arteries at physiological distension pressures following selective destruction of either protein. Arteries exposed to elastase dilate aneurysmally

and become stiffer as collagen is recruited, but do not rupture. If exposed to collagenase, vessels dilate slightly, and tend to rupture at low pressures. These findings suggest that under physiological conditions, elastase is responsible for maintaining the normal dimensions of the artery, whilst collagen provides the tensile strength.

Removal of appreciable proportions of either structural protein will weaken the aortic wall, and might be expected to predispose to aneurysm formation.

Elastin Destruction

Elastin is a stable protein, with a slow turnover in adult humans, having a half life estimated at 70 years.[147] Most elastin is synthesised early in the postnatal period and is not replaced. With increasing age, elastin degenerates and is removed from tissues, a process responsible for the ageing changes in skin, and for age related aortic dilation.

Aortic aneurysm formation is histologically associated with marked elastin loss. The degree of elastin loss is greater than in non-aneurysmal aorta displaying the same degree of atheromatous change, implying that the exaggerated elastin loss is related specifically to the presence of aneurysm disease.[148]

Elastase activity is normally extremely small in the peripheral blood in adult humans. Circulating elastase normally exists as an inactive complex, principally with alpha-1-antitrypsin. Decreased levels of alpha-1-antitrypsin (a-1-at) or its activity leads to increased free elastase activity.[149]

Smoking has been noted to reduce antitrypsin levels by two main mechanisms. Oxidants in cigarette smoke oxidise methionine residues on alpha-1-antitrypsin, inactivating it. Inhibition of a-1-at by neutrophil myeloperoxidase has also been described.[150,151]

Cannon and Read investigated elastase activity in peripheral blood in 1982.[152] They suggested that in patients with aneurysm, serum and neutrophil elastolytic activity was increased, and antiproteolytic activity reduced, compared with patients with aorto-iliac occlusive disease. The validity of the data in their paper, was marred however by small numbers and dubious statistical significance values. Attention was drawn to the importance of homeostasis between elastase activity and its inhibition by antiproteolytic agents, such as alpha-1-antitrypsin.

Hornebeck et al separated the total elastase activity in human serum into that due to serine proteases, such as neutrophil elastase, and metalloproteases, but found no increase in total elastase activity in atherosclerotic patients compared to normal controls.[153] Contradictory findings were made by Brown in 1985, who, using the same assay, found raised levels of serum proteolytic activity in patients with aorto-iliac occlusive disease and aneurysms compared with controls.[154] Like Hornebeck, Brown found that human serum could not hydrolyse insoluble bovine elastin, and it is likely that the new proteolytic enzyme that Brown describes is the metalloprotease component originally described by Hornebeck.

Endopeptidases capable of hydrolysing insoluble elastin have been isolated from various tissue types, including pancreas, spleen, neutrophil polymorphs, macrophages, fibroblasts, and vascular smooth muscle cells.[155-157] Hornebeck et al. in 1976 correlated increasing aortic elastase activity with increasing age and arteriosclerosis.[158] They went on to isolate an elastase-like

enzyme from human aorta immunologically distinct from human pancreatic or neutrophil elastase, which was responsible for 36% of total elastolytic activity in the wall.[159]

Further studies of aortic tissue elastase activity were undertaken by Busuttil in 1982. His group demonstrated an increase in total elastase activity in the walls of aneurysmal aorta compared to atheromatous, non-aneurysmal aorta.[160] The findings of increased elastase activity in aneurysmal aortic wall were confirmed by Cohen in 1987, who also reported an increase in elastase in ruptured aneurysmal aortic wall.[161] Cohens group went on to characterise the elastase in aneurysmal aortic wall, concluding that it was a neutral serine protease. In view of the findings of Hornebeck, ie. that there are clearly more than 1 elastases in aortic wall, it is likely that the aortic wall extract used by Cohen contained a heterogeneous mix of serine elastases. Despite this, and to the detriment of the work, he compares his rather crude aortic wall elastase extract to the elastase extract used by Hornebeck. This was an aortic protease refined by affinity chromatography, and therefore the comparison is invalid. The elastase used by Cohen had similar properties to smooth muscle elastase isolated from rat and pig smooth muscle cells. This identification lacked immunological proof, but may indicate that at least some part of the elastase activity of the aneurysmal aortic wall may be produced by smooth muscle cells.

An excellent and recent review of the state of research into the enzymatic basis of aneurysm disease can be found in the paper by Powell and Greenhalgh in 1989.[162] One point made in this review is that in inflammatory aneurysms, 30% of the elastase which can be precipitated by antibodies to human neutrophil elastase. In the case of non-inflammatory aneurysms, 90% of the elastase is *not* precipitated by this antibody. The problems remain, however, that in established aneurysm disease, the presence of increased

elastase activity in either serum or aortic tissue may be a cause or an effect of the aortic dilation.

Collagen Destruction

The part played by collagen in aortic function had already been outlined. Collagenase was first detected in aneurysmal aortic wall by Busuttil in 1980.[163] It appeared from this study that collagenase activity was limited to the walls of aneurysms, and was not present in the walls or atheromatous, non-aneurysmal aorta. Collagenase activity correlated to aneurysm size, and was particularly high in the single ruptured aorta studied. In cases where the location of maximum collagenase activity within the wall was studied, this was found to be in the inner layers of the wall.

Discovery of collagenase in the wall of aortic aneurysms and its correlation with size tends to confirm the findings of a number of other authors. The work of Dobrin referred to above in the mechanical role of collagen, and his findings in arteries exposed to collagenase related rupture with loss of collagen. As rupture also correlates with size, the correlation of collagenase activity with size (and rupture) is significant.

Collagenase activity localised to the inner layers of the aneurysm wall confirms the findings of Strandness and Sumner that collagen is concentrated in the outer layers.[164] The finding of collagenase also tends to confirm the findings of Swanson that rupture of aneurysms is commoner following laparotomy, owing to a proposed but unproven increase in systemic collagenase.[165]

In addition to confirming other authors findings on increased collagen to elastin ratios in aneurysmal aorta, Menashi suggested that low levels of type 3 collagen may be important in the genetic predisposition to aortic aneurysms seen in some families.[166] Deficiency of type 3 collagen has been shown to be associated with arterial aneurysm formation in Ehlers Danlos type 4 and in some patients with cerebral aneurysm formation.[167,168] It may be then that alterations in the quality as well as the quantity of collagen may be implicated in the formation of aneurysms.

Defects in Elastin and Collagen Formation

Defects in both collagen and elastin have been linked with deficiencies in trace elements such as copper, zinc and magnesium.[169,170] The biochemical basis of these theories has been extensively investigated, but their place in the pathogenesis of aneurysms in humans is less clear.

The mechanical properties of both collagen and elastin are due to extensive covalent cross-linking between protein chains within each fibre. These cross-links are formed between lysyl residues present on adjacent chains by the action of lysyl oxidase. Copper is an essential cofactor in lysyl oxidase[171], and therefore in the formation of normal collagen and elastin. Copper deficiency has been associated with aneurysm formation and aortic rupture in lathyritic turkeys, blotchy mice, swine and rats. Tilson has found that the collagen formed in the congenitally copper deficient blotchy mouse is abnormally soluble because of reduced collagen cross-linking. This animal model also displays reduced activity of lysyl oxidase, in keeping with the copper deficiency. A human model of lysyl oxidase deficiency is available, documented by Di Ferrante, in Ehlers-Danlos Syndrome type 5.[172]

A number of studies in humans have associated aneurysms of the abdominal aorta with low copper levels in various tissues.

More recently, Tilson has refuted his own, earlier findings, and Senapti has failed to confirm Tilsons original findings on hepatic copper levels in aneurysm patients.[173-176]

The only recognised defect of copper metabolism in humans is Menkes Syndrome. This rare genetic defect leads to an abnormality in copper absorption and transport, and the formation of aneurysms. In view of the other findings outlined above, it is likely that in man, copper deficiency never reaches such severity that it plays a significant part in abdominal aortic aneurysm formation.

Copper deficiency has however been implicated in other mechanisms which may lead to aortic damage. During normal electron transport to molecular oxygen in the metabolic respiratory chain, toxic partial reaction products are formed which cause damage to many biomolecules. Such products include oxygen free radicals. Superoxide dismutase is an enzyme responsible for the conversion of oxygen free radicals to water and molecular oxygen, and which therefore protects against tissue damage from free oxygen radicals. Copper is an essential cofactor in superoxide dismutase, without which the enzyme will not function. In the avian model, copper deficiency leads to marked reduction of superoxide dismutase activity, which is reversed by the administration of copper in the intact animal. It is conceivable that severe copper deficiency in humans could lead to reduced superoxide dismutase activity, and a rise in levels of tissue-toxic free oxygen radicals. [177]

4. Genetic Associations

The genetic association of aortic aneurysms in the animal model has been recognised for some time.[171,178] The predisposition to aneurysm formation in blotchy mice is closely linked to copper metabolism. This mechanism has not been confirmed in humans, except in the extremely rare condition of Menkes Syndrome. It is of doubtful significance in the genesis of non-specific aneurysm disease in man.

A familial tendency to aortic aneurysm formation was first proposed by Clifton in 1977.[179] He described the familial clustering of ruptured abdominal aortic aneurysms. Following this report, the genetic predisposition to aortic aneurysm formation attracted little attention until the mid 1980's.

Tilsons studies of the genetics of aortic aneurysms in 1984 were strongly suggestive of a genetic basis for aneurysm disease. There was inconsistency in the mode of transmission, some families displaying strong evidence of X-linkage, and some of an autosomal pattern. The point is made by Tilson that this situation is not unique, and that differing genotypes may give rise to the same phenotype in other human diseases.[180] Tilson also noted an association of carcinoma and aortic aneurysm, which he explains on the basis of changes in tissue matrix consequent upon inherited abnormalities in collagen metabolism.[181]

The finding of familial clustering was confirmed in later work with greater numbers by Norrgård and Johansen, but the likelihood of genetic heterogeneity remained.[182-184]

The phenomenon of sex predisposition must be accounted for, and X-linkage of a relevant susceptibility gene would provide at least a partial explanation. Williamson and others have found changes in collagen cross linking in animal models attributable to modulation of lysyl oxidase activity by sex steroids,[185,186] and the potential mechanism has been described above.

Genetic variations in the activity of protease inhibitors have also been implicated in aneurysm disease. The two most thoroughly investigated are alpha-1-antitrypsin (a-1-at) and Tissue Inhibitor of Metalloproteases (TIMP).

As the major inhibitor of neutrophil elastase, the activity of a-1-at is genetically determined. The possible relevance of protease inhibitor deficiency has been stated above, but there is conflicting evidence as to the role of heredity. Tilson has documented a deficiency allele phenotype of a-1-at in 3 of 13 patients with aortic aneurysm, while Powell has detected only 1 such patient in 50.[187-188] While manifest a-1-at deficiency may be a cause of a small and defined subset of aortic aneurysms, it clearly cannot account for the majority. Neither would it account for male sex predominance, as the a-1-at gene is autosomal.

TIMP is a recently discovered and characterised antiprotease present in human tissues. It is a large and highly stable molecule, and has been identified as a major inhibitor of at least three connective tissue proteases: Collagenase, gelatinase and proteoglycanase.[189,190] TIMP has been demonstrated in aortic tissue,[191] and its gene has recently been assigned to the X-chromosome.[192] There may be some evidence to suggest that patients with aortic aneurysms demonstrate a relative deficiency in TIMP in skin biopsies, but research in this field is at an early stage.[191]

SUMMARY

Aortic aneurysms are associated with age and atheroma. Both of these factors predispose to aortic dilation and decreased aortic compliance because of changes in structural proteins in the aortic wall. Initial changes in aortic diameter and compliance may predispose to continued dilation, as increased pulse wave reflection from the aortic bifurcation leads to increased aortic stressing.

The structure of the abdominal aorta in man is such that it sustains a disproportionally high tension per medial lamellar unit in the face of an almost total absence of vasa vasora. Because of a lack of 'reserve' capacity in mural blood supply, occlusion of a few vasa vasora by atheroma may lead to medial degeneration and predispose to aortic dilation.

Loss of the structural proteins elastin and collagen has been associated with aneurysmal dilation and rupture of the aorta.

Increased elastase activity has been found in aneurysm wall, and is due to a heterogeneous group of elastase enzymes. Neutrophil elastase has been associated with inflammatory aneurysms, in which it constitutes some 30% of total elastase activity. Increased elastase activity is also present in the peripheral blood of patients with aneurysms.

Collagenase activity is raised in tissue from aneurysm wall, and is correlated with size and rupture.

Decreased levels of protease inhibitors have been found in the wall and peripheral blood of patients with aneurysms.

Two mechanisms implicating Copper deficiency in aortic aneurysm formation have been proposed. Reduction in activity of the copper dependent enzymes lysyl oxidase, and superoxide dismutase. These mechanisms have been strongly associated with aneurysm formation in animal models, and in very rare conditions in humans. It is highly unlikely that copper deficiency develops in adult humans to such a degree that either of these mechanisms are material in the formation of aneurysms of the aorta.

CHAPTER 3

INFLAMMATORY ANEURYSMS

INTRODUCTION

The early history of inflammatory aneurysms, and its emergence as a distinct clinical and pathological entity have been dealt with in Chapter 1. This chapter will summarise the pathological features of inflammatory aneurysm, including current theories of ætiology and will go on to discuss the clinical features and treatment options in inflammatory aneurysm disease.

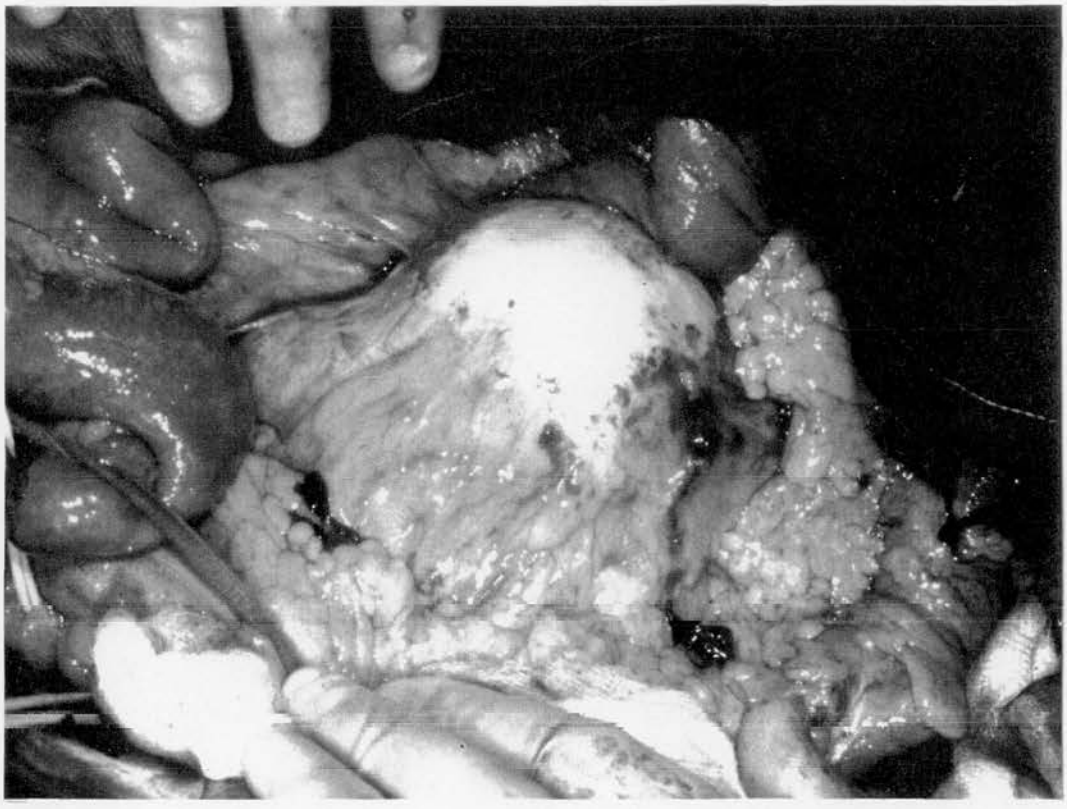


Figure 10.

Inflammatory aortic aneurysm. The thick white fibrous coat and adherent viscera can be seen.

1. Pathology

Inflammatory aneurysms make up 2.5%-23% of all aortic aneurysms.[193,194] The condition, like simple atherosclerotic aneurysms of the aorta, is more common in males, but occurs in a population approximately a decade younger.[195,196] Inflammatory change is usually confined to the infrarenal aorta, but cases of inflammatory aneurysm of the thoracic aorta and iliac arteries have been reported.[193,195,197] The characteristic finding is of an aortic aneurysm covered on its anterior and lateral aspects in a thick pearly white fibrous coat. This fibrosis may extend laterally in the retroperitoneum to encase a number of retro-peritoneal structures, such as ureters, renal vessels, duodenum, inferior vena cava, small bowel mesentery and sigmoid colon.[103,105,195,198]

Macroscopically, inflammatory aneurysms present a pearly white appearance. The wall is extremely thick, and may exceed normal thickness by 3 or 4-fold, the excess fibrotic thickening being external to the media.[195]

Microscopically, there are features of severe atheromatous disease, with ulcerated fibrolipid plaques, and intimal proliferation and fibrosis. There is marked disruption and loss of the internal elastic lamina. The media is extremely attenuated and fragmented, with loss and fibrous replacement of smooth muscle cells and elastic fibres. The adventitia is thickened and infiltrated by chronic inflammatory cells, lymphocytes and plasma cells, so intensely in places that germinal centres are formed. This infiltrate often extends into the media and obscures the demarcation between media and adventitia. There is frequently severe inflammatory endarteritis of adventitial and medial blood vessels. Adjacent peripheral nerve fibres, lymph nodes and other structures may be seen entrapped in the adventitial fibrosis. Findings by

a number of authors are remarkably consistent.[102,106,195,197]

Figure 11a.

Lymphocytic infiltrate in the mid-media of an inflammatory aneurysm.

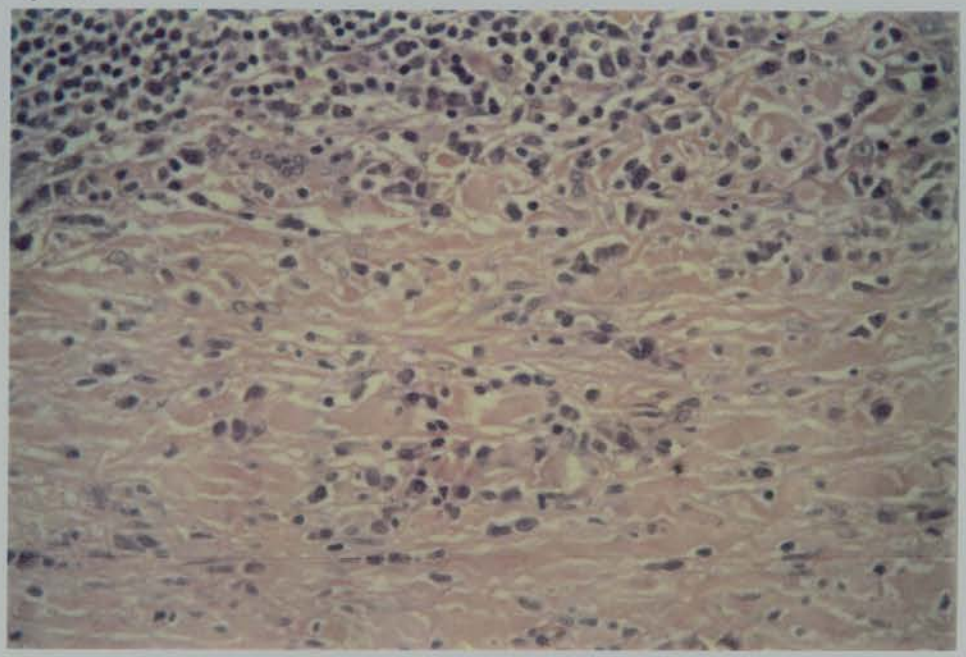


Figure 11b.

Early germinal centres in the wall of an inflammatory aneurysm.

Figure 11c.

Perivascular infiltrate and haemosiderin laden macrophages in the wall of an inflammatory aneurysm.

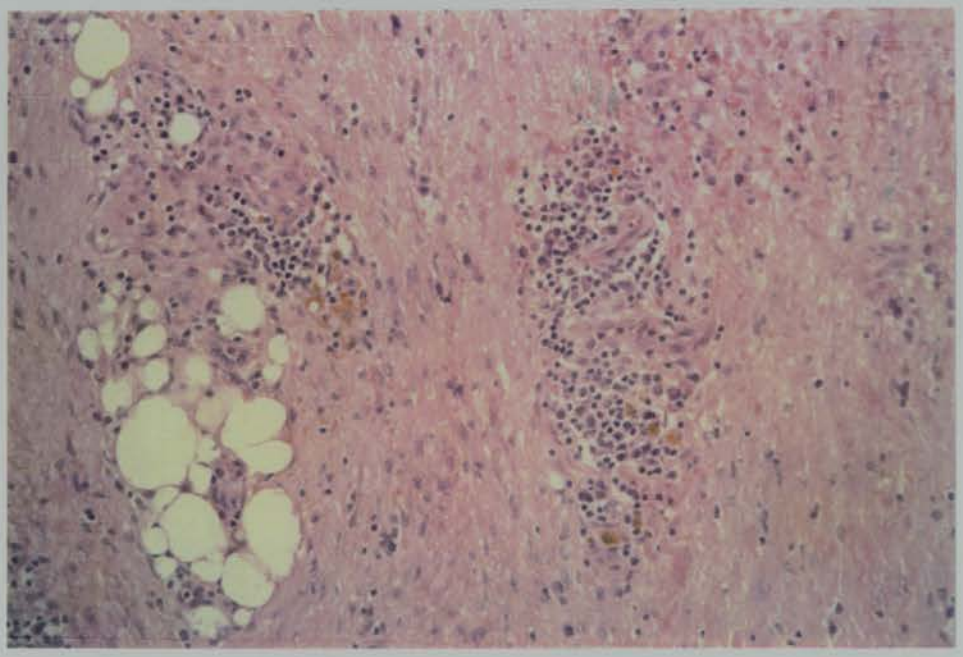


Figure 11d.

Perineural infiltration with plasma cells and lymphocytes in the adventitia of an inflammatory aneurysm.

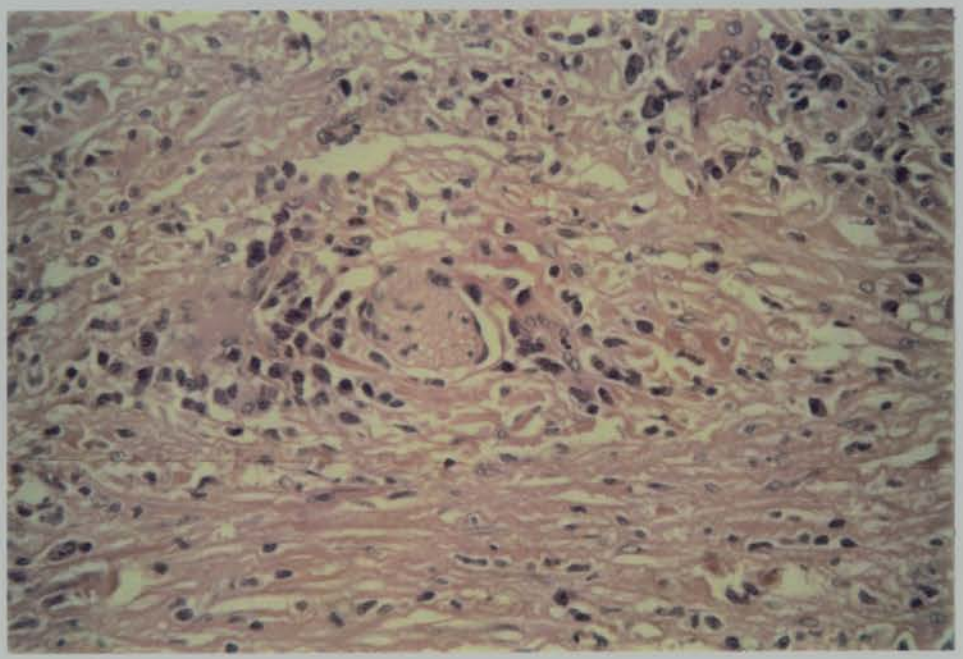


Figure 11e.

Giant cells in the wall of an inflammatory aneurysm.

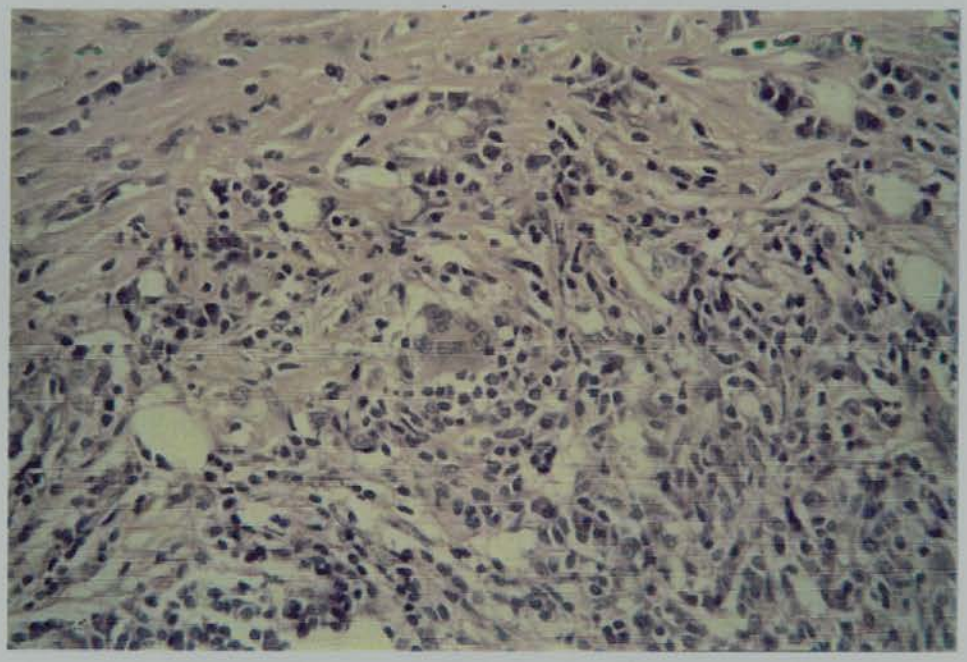
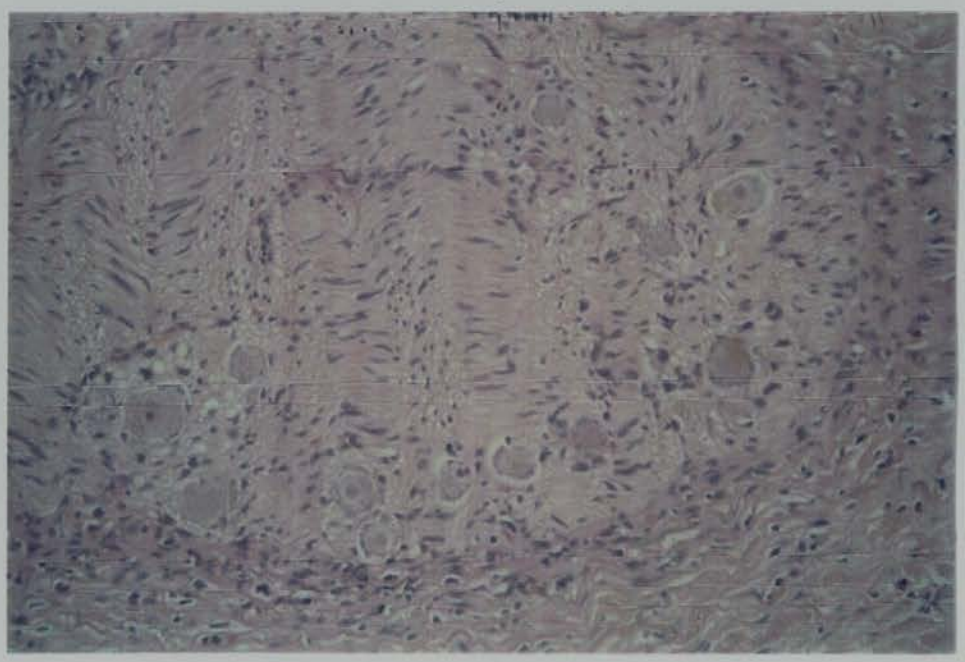


Figure 11f.

Periaortic sympathetic ganglion trapped in inflammatory fibrous tissue.



Approximately three quarters of aortic aneurysms exhibit some degree of inflammatory cell infiltrate and adventitial fibrosis, a finding absent from Goldstone's series, but confirmed by a number of authors including Rose & Dent and Mitchinson. Aneurysms which display the macroscopic features of inflammatory aneurysm, and are classified as such, show an excessively intense cellular and fibrous response. In their extensive work, Rose and Dent (1981) and Mitchinson (1984) conclude that inflammatory aneurysm is the result of atherosclerosis, and that it represents the extreme end of a spectrum of disease.[106,197]

The precise ætiology of inflammatory aneurysm is unknown, although several theories have been proposed. The two most commonly proposed theories are:

1. That aortic aneurysms develop, and go on to become inflammatory, developing the typical macroscopic, clinical and histological features of inflammatory aneurysm.

i.e. *Aneurysm causes fibrosis*

2. That the inflammatory response develops around the abdominal aorta, which then causes aneurysm formation.

i.e. *Fibrosis causes aneurysm*

Most authors favour the former possibility, in that it can account for two phenomena associated with inflammatory aneurysm. These are listed below.

1. The presence in some cases of inflammatory aneurysm of localised plaques of mature fibrosis, rather than the more complex 'frozen' aorta with multiple organ involvement.

2. The existance of an intermediate type of aneurysm, in which organs are adherent and difficult to dissect, but without the presence of plaques of mature fibrous tissue.

It does not however, account for the existance of non-aneurysmal periaortic fibrosis, which is most often seen as part of idiopathic retroperitoneal fibrosis (IRF).

A common aetiology has been proposed for IRF and inflammatory aneurysm, but there seems little doubt that inflammatory aneurysm is a distinct pathological entity in its own right. In both Crawfords and Goldstones series, they are described as "a close relative, but distinct clinical entity" and "a distinct clinical entity"[103,193] The gross distribution of fibrosis differs; in IRF the fibrosis is maximal over the sacrum,[193,199] whilst in inflammatory aneurysm, fibrosis is often confined to the aneurysm wall itself,[193] with no other distribution in the abdomen.

Inflammation has been noted by several authors to be related to severe atheroma, and changes localised to sites of medial breaching by atheromatous plaques.[200-202] The concept of inflammatory aneurysm as a separate entity is supported by a number of authors.[193,197,203]

Leakage of blood components has been proposed as an important factor in the ætiology of inflammatory change. No studies, however, have shown either the leakage of fibrin or haemosiderin to be consistently present in large series.[106,195,204]

Although Goldstone[103] acknowledges the presence of characteristic gross and microscopic features in inflammatory aneurysm, he regards the inflammatory response as nonspecific, and attributes no ætiological agent. West & Ryan[205] report a case suggesting an athero-ischæmic ætiology.

Currently, the most credible and popularly accepted theory of the ætiology of inflammatory aneurysm is that proposed by Mitchinson. In a number of publications, he suggests that the cellular and fibrous response seen in inflammatory aneurysms is due to an immune response to antigen breaching the attenuated and eroded media. The antigen is thought to originate in atheromatous plaque, and may be related to oxidised lipoproteins.[197,201,202] The role of immunological mechanisms has also been anticipated by Munro et al., who found the presence of immunoglobulin A - bearing plasma cells in aortic aneurysm wall increased in proportion with aneurysm wall thickness.[206]

If immunological hypotheses prove correct, the theories of ætiology of inflammatory aneurysm could be unified:

(a) Atherosclerosis as the root cause of the aneurysm.

(b) Inflammatory change in the wall of the aneurysm dependant upon the severity of the atheroma, and therefore on the degree of antigen presentation. This would explain the presence of mild inflammatory infiltrate in 'simple' aneurysms, and the impression of many authors of a spectrum of disease.

(c) Clinically recognised inflammatory aneurysm resulting from severe atheroma, large volume of antigen presentation, and correspondingly excessive cellular and fibrous response.

2. Clinical Features of Inflammatory Aneurysms

There is remarkable consistency in the description of clinical features of inflammatory aneurysms between all the major reviews. Pain in the abdomen or back is an extremely constant finding,[102,103,193,195,198,207,208] and may be so severe as to be mistaken for a leaking aneurysm. The pain may be caused by vertebral erosion, or by visceral obstruction by the inflammatory mass,[102,195,207]

which may lead to the patient being treated by urgent operation. [193,196,199]

Weight loss is a frequent complaint,[193,195,207] and there may be signs of systemic illness [207]. On examination the aneurysm is often very tender in the absence of rupture or leak.[193]

3. Laboratory Investigation

The most common finding on haematological examination is of a raised ESR [102,198,207,208], and anaemia has been noted in some series.[198] The white blood count is usually normal.[106] Obstruction of the ureters may lead to a raised blood urea.[193]

4. Radiological Diagnosis

The radiological diagnosis of inflammatory aneurysm is a subject of considerable controversy, and most of the currently available modalities have been assessed. The diagnosis of aneurysmal change and inflammatory change are closely linked. Because of this, and because radiological diagnosis forms a large part of this thesis, radiological techniques of diagnosis are discussed in detail in the following chapter.

5. Operative Findings

The extensive fibrosis associated with inflammatory aortic aneurysms frequently requires extensive modifications of surgical technique during aneurysmectomy. In florid cases, the left renal vein, inferior vena cava, ureters and duodenum may be included in the peri-aneurysmal mass of

fibrosis. The incidence of duodenal involvement in fibrosis has been reported as 62-100%,[209,210] with involvement of the inferior vena cava almost as frequent.[103,209]. The ureters are drawn into the peri-aortic fibrosis less frequently, the incidence ranging from 12% to 30%.[207,210] The fibrosis renders dissection of the neck of the aneurysm for cross-clamping hazardous, with a high risk of damage to other organs.

6a. Operative Technique - Exposure and resection.

Several factors have precluded the extensive study of operative techniques in inflammatory aneurysm. Not only is the recognition of inflammatory aneurysm relatively recent, but also the incidence is low, and current radiological diagnostic accuracy is poor.

Most surgeons, even those with a specific vascular interest therefore see relatively few of these aneurysms and the diagnosis is unlikely to be made pre-operatively. In these circumstances, a detailed plan for operation cannot be made, and the operative mortality for inflammatory aneurysm is much higher than for simple aneurysms.

In all of the substantive reviews of inflammatory aneurysms where operative technique has been considered, aneurysm repair has been undertaken by the transperitoneal route. Several studies report cases where operation has been abandoned because of the extent of fibrosis, with resort to alternative methods of treatment. The incidence of abandonment however is low in most series, ranging from none[203] to 60% (three of the five cases reported by Stringer).[196] Crawford's series[193] includes 10 patients who, having undergone laparotomy elsewhere, were referred for operation. In all cases

this was successfully performed. In the small series reported by Stringer, two of the three patients had radiological features on intravenous urography suggestive of inflammatory aneurysm, but no other radiological investigation apart from ultrasound was performed.

Analysis of the results from several large series show the overall incidence of abandoned operation to be 4.2%.

In exposing the neck of the aneurysm for cross-clamping, most authors agree on a technique involving minimal dissection of adherent viscera, particularly the duodenum and inferior vena cava. Both Crawford's and Pennell's series include patients dying from graft infection secondary to duodenal injury at the time of injury. No attempt should be made to separate any viscus from the inflammatory mass, and proximal control of the aneurysm should be gained in an area free from fibrosis. In most cases, the supraduodenal aorta is relatively uninvolved in fibrosis and permits clamping.[195,209] Where this is not the case, and fibrosis extends to encase the left renal vein, renal arteries and supra-renal aorta, the alternatives are to abandon the operation for non-operative treatment,[196,208,209] or to extend the incision to gain access to the aorta more proximally. Crawford gives a comprehensive description of the techniques of aortic clamping in the intra-abdominal/suprarenal and thoracic sites, and reports good results.[193]

More recently, the retroperitoneal approach has been advocated for routine use in cases of inflammatory aneurysm.[211] Although the advantages of this approach are controversial in cases of simple aneurysm,[212,213] it may be useful where there is marked inflammatory change. The planned use of the retroperitoneal approach for all inflammatory aneurysm assumes reliable pre-

operative diagnosis. As will be demonstrated in the following Chapter, current radiological techniques are incapable of providing this level of diagnostic accuracy.

6b. Operative technique - Ureterolysis.

The necessity of a minimal dissection technique in exposure of the aorta seems clear, but the need for operative ureterolysis is less so. The ureters are frequently drawn medially by fibrosis, and may be difficult to recognize. Sterpetti and Pennell both advocate catheterisation of the ureters pre-operatively to aid in identification, and avoid the small but documented incidence of ureteric damage.

Study of the 212 patients included in the studies by Sterpetti, Goldstone, Baskerville, Pennell and Crawford, reveals that 52 were noted at operation to have ureteric involvement in the inflammatory mass, an overall incidence of 24.5%. 32 patients underwent ureterolysis, either on one side or both, and had a satisfactory outcome. In fourteen patients, no action was taken apart from prosthetic replacement of the aneurysm, again with an apparently satisfactory outcome in all cases. Three patients were treated with steroids rather than laparotomy (Baskervilles series); all had resolution of radiological signs of ureteric involvement. Of the remaining three, two sustained ureteric damage at operation, proceeding to ligation of the ureter, and one had a nephrectomy for advanced obstructive damage and recurring infections.

There seems therefore to be a case for expectant management when the ureters are involved in the fibrosis surrounding inflammatory aneurysm. Ureterolysis should be reserved for cases of hydronephrosis. Not only does this avoid increased blood loss, as highlighted by Goldstone, but it also prevents the incidence of ureteric damage reported by Pennell, an enthusiastic advocate of ureterolysis.

Cases of ureteric obstruction which continue or occur de novo following replacement of inflammatory aneurysms are rare. Both Pennell[195] and Crawford[193] comment that this has never been reported, and in Sterpetti's later paper in 1989 he comments that only two were found on review of the literature.[203] One such patient not quoted by Sterpetti was reported by Baskerville in 1987.[214] Their patient underwent aortic replacement for a typical inflammatory aneurysm, but presented 4 months later with bilateral ureteric obstruction. The obstruction resolved following treatment with steroids. There is therefore a paucity of direct experience in the treatment of failed conservatism in post-operative ureteric obstruction in inflammatory aneurysm disease. With the apparent success of pre-operative steroid treatment in ureteric obstruction, the little evidence available suggests that, where conservative therapy fails, steroids may be used post-operatively to relieve ureteric obstruction.

Non-Operative Treatment

Steroid treatment has been used both as an adjunct to laparotomy, and as a sole treatment for inflammatory aneurysm. In the series reported by Clyne in 1977[215] and by Baskerville in 1983[208] corticosteroids were used to reduce the fibrotic reaction surrounding inflammatory aneurysms which were

deemed inoperable following investigation or at laparotomy. All 7 patients in these two studies recovered well. Unfortunately Baskerville had the opportunity to re-examine only one of their series at laparotomy following steroid treatment. It does seem reasonable, however, to suppose that steroid treatment can be used to reduce the inflammatory mass, and expedite operation in cases where facilities or expertise are limited.

There is no real evidence to suggest, however, that routine primary steroid treatment of inflammatory aneurysms is necessary. Sterpetti has proposed that it may in fact be theoretically dangerous, and predispose to acute rupture by thinning of the fibrotic wall.[203] Evidence to lend support to this hypothetical possibility is provided by Baskerville's findings of marked thinning of the inflammatory aneurysm wall following steroid treatment.[208]

SUMMARY

Inflammatory abdominal aortic aneurysm is a distinct clinical entity, which frequently presents as a painful tender aneurysm in younger patients. Patients may also complain of weight loss or features of a systemic illness. The aortic aneurysm is associated with peri-aortic fibrosis, displaying severe destruction of the formed elements of the wall and a marked chronic inflammatory cell infiltrate. The fibrotic reaction may include and encase adjacent organs, and may render operative treatment hazardous.

Operative techniques are described to minimise difficulty and morbidity, but their planned use depends upon the accurate pre-operative diagnosis of inflammatory aneurysm and assessment of its extent. Currently, diagnostic accuracy is poor, and the diagnosis of inflammatory aneurysm is often made first at laparotomy, leading to a small incidence of abandoned operation because of 'inoperability' and an increased morbidity and mortality where reconstruction is attempted.

The primary treatment of choice for inflammatory aneurysms remains operative replacement, there being no well defined place for steroids in this role. Despite this, there is evidence that steroids may be useful in highly selected patients as an adjunct to operation.

Operative exposure of inflammatory aneurysms should be performed using a minimal dissection technique, and operative ureterolysis retained for cases of hydronephrosis. An alternative retroperitoneal approach is an advantage where the inflammatory process extends proximally to involve the renal vessels and duodenum.

CHAPTER 4.

RADIOLOGICAL INVESTIGATION OF AORTIC ANEURYSMS, WITH SPECIAL REFERENCE TO INFLAMMATORY ANEURYSMS.

INTRODUCTION

The radiological diagnosis of inflammatory aneurysms is closely linked with the diagnosis of simple aneurysm disease. This chapter summarises the ability of currently popular radiological techniques to provide information necessary to the clinician in both simple and inflammatory aneurysms.

An appraisal of the abilities and current uses of MRI is included. The principles of MRI are outlined in a brief technical synopsis, and the rationale for the screening sequences used in this study are discussed.

The principal requirements of any radiological investigation used in aortic aneurysm disease are that reliable and reproducible visualisation can be achieved of the following:

1. The presence of aneurysmal dilatation of the aorta.
2. The upper extent of aneurysmal dilation, and its relationship to other vessels, particularly the renal artery origins.
3. The diameter in both anteroposterior and transverse dimension of the main body of the aneurysm.
4. The thickness of the aneurysm wall, and the retroperitoneum of the mid-abdomen.

Following a discussion of the exact radiological definition of an aneurysm, each of these will be considered in turn, and the abilities of conventional radiology reviewed for each.

1. The definition and measurement of aneurysmal aorta.

The precise definition of an aneurysm of the abdominal aorta remains unresolved, and there is no universal agreement. Working criteria have been used by some authors in population screening. Allen et al. consider an aneurysm to be present when the diameter of the infrarenal aorta is greater than 3 cm., or 5 mm greater in diameter than the suprarenal aorta.[216] These criteria are also used by Collin, but the upper limit of the infrarenal aorta is extended to 4 cm.[217] The ratio of infra to supra renal diameter[218] has been used in the study of aneurysm growth rates.

Aneurysm measurement may be made in either transverse diameter or anteroposterior (AP) diameter. Transverse diameter is measured clinically, but AP diameter is more accurately measured on ultrasound than transverse diameter.[219] Cronenwetts actuarial analysis suggests that the initial measurement of AP diameter is a better predictor of eventual outcome than the transverse diameter or the ratio between the diameters of the aneurysm and the proximal aorta.[220]

The diameter at which operative treatment of small aneurysms should be recommended is currently the subject of a National trial.

2. The presence of aneurysmal dilatation

In the past, aortography has been used in the assessment of both the size and extent of aortic aneurysms.[221-224] It has been found to be a reliable method for aneurysm diagnosis, detecting over 93% of aneurysms in Gordon-Smiths series[225] and up to 100% in other series such as that of Brewster et al.[224], but many authors have been unable to match these results.[226,227] Whilst Rich et

al. and Brewster[224,228] advocate routine angiography in aneurysm disease, others favour its selective use.[229,230] Conventional aortography is highly invasive, and associated with a number of complications. This, and the development of other modalities, such as ultrasound, have rendered conventional angiography obsolete as a method for the initial diagnosis of aortic aneurysm.[227,231] Ultrasound is a cheap, highly accurate, and completely non-invasive examination and confirms the presence of aortic aneurysm in well over 95% in all series.[232]

3. The upper extent of the aneurysm, and its relation to the renal arteries

The level of the upper extent of the aneurysm and its relation to the renal arteries is well shown by aortography in most authors hands using both conventional aortography and intravenous subtraction techniques.[230,233] The presence of double renal arteries is also demonstrated in the majority of cases,[233] as are aorto-caval fistulæ and aortic dissection.[234,235] Inflammatory aneurysms are notoriously poorly defined on aortography.[236]

Failure to reliably show the upper end of aortic aneurysms is the main limitation of ultrasound. Diagnosis of the level of the renal arteries is often made by inference, using the level of the superior mesenteric artery as a reference point. Overlying bowel gas prevents the visualisation of the aneurysm neck in the majority of cases.

Computerised tomography (C.T.) combines the abilities of both aortography and ultrasound. It is less invasive than angiography in its use of ionizing radiation and intravenous contrast media. Aneurysm detection by C.T. approaches 100%, but demonstration of the relationship between renal

arteries and the upper end of the aneurysm may be unreliable.

Renal arteries are not infrequently seen on C.T. scans for aneurysm, but some authors suggest that the *origins* of the arteries are less easily seen.[233] Other studies report excellent results with contrast enhanced C.T. in the diagnosis of renal artery involvement, similar to that of angiography.[226,237] In their recent paper, Vowden et al.[230] report considerable underestimation of the level of the renal arteries, and overestimation of the incidence of juxta or supra renal aneurysm extension using contrast enhanced C.T. They attribute this to aortic buckling and cephaloid extension of the aneurysm sac.

It would seem appropriate to conclude that in the investigation of involvement of the renal arteries and their relationship to the upper end of aortic aneurysm, the most accurate investigation is aortography. Recent evidence suggests that intravenous D.S.A. provides as much anatomical information as the conventional technique, whilst obviating some of the (infrequent) complications thereof.

4. Aneurysm size

Ultrasound has been shown consistently to be accurate in measuring aneurysm size in a number of studies,[226,227] though recently doubt has been cast on its reproducibility.[238] Its cheapness and non-invasive nature have made it the method of choice in the initial confirmation of clinical findings in aneurysm disease. Similarly the ability of contrast enhanced C.T. to demonstrate aneurysm size is largely established and undisputed.

Aortography by any technique displays only lumenal diameter: It may suggest the diagnosis by demonstration of an ectatic lumen but objective measurement of aneurysm size is impossible by this method.

5. Aneurysm wall thickness and retroperitoneum

Assessment of both of these characteristics is of particular relevance to the diagnosis of inflammatory aneurysm and will be discussed in this context.

Intravenous urography has been used to demonstrate the medial indrawing of ureters by peri-aneurysmal fibrotic change. This is, however, a non-specific sign, and only accurate in 30% of cases of inflammatory aneurysm.[195]

Aortography is notoriously unreliable in showing peri-aortic tissues and cannot be used for the diagnosis of inflammatory aneurysms.[236] It may however produce a urogram of some diagnostic value.[193,203]

Considerable variability exists in estimates of the ability of ultrasound to detect inflammatory aneurysms. Characteristic findings of an echo-free peri-aortic halo anterior to an enlarged aorta were reported by Henry et al. in 1978,[239] and by Cullenward in 1986.[240] These have, however, been inconsistent and infrequent diagnostic signs. Cheatle et al.[210] failed to make the diagnosis of inflammatory aneurysm by ultrasound in all 4 of their cases, as did Stringer & Bentley[196] in their series of 5, and Plate et al.[198] in their series of 6. Fitzgerald and Blackett in a retrospective review of 17 patients quote an ultrasound detection rate of only 22%,[241] and there is now general consensus that ultrasound is of little value in detecting inflammatory

change.[196,198,203]

The presence of inflammatory aneurysm may be suggested by the demonstration of ureteric obstruction and hydronephrosis in the presence of an aortic aneurysm. Pennell et al.[195] documented an incidence of hydronephrosis in 18% of their series of 127 patients with inflammatory aneurysms.

Computerised tomography has been the only reliable radiological method available for the direct diagnosis of inflammatory aneurysms. The diagnosis and characteristic signs of inflammatory aneurysm were first reported by Ramirez in 1982,[242] and by the mid 1980s, C.T. was the radiological diagnostic method of choice.

Vint et al.[243] and Johnston et al.[244] also comment on the characteristic signs of inflammatory aneurysm on C.T. scan. Subsequently, C.T. has become the gold standard for the diagnosis of inflammatory change. In Cheatles series however, C.T. proved useless for pre-operative diagnosis. This is somewhat surprising, as other studies suggest figures ranging from 41% in the prospective part of Fitzgerald and Blacketts study to 50% (Pennell et al.) and 100% (Plate et al., Crawford et al.,). It should be noted that Plates series was of 5 patients, and 6 of Crawfords 10 patients had had the diagnosis of inflammatory aneurysm made by laparotomy before C.T. scanning was performed. In reality then, the diagnostic accuracy of C.T. in large series rarely exceeds 50%. No series has been able to match the 100% accuracy of C.T. diagnosis from Baskerville's series in 1983.[208]

Figure 12.

Ultrasound scan of inflammatory aortic aneurysm. The thickened wall can be seen (arrow). Thrombus is present within the lumen (L).

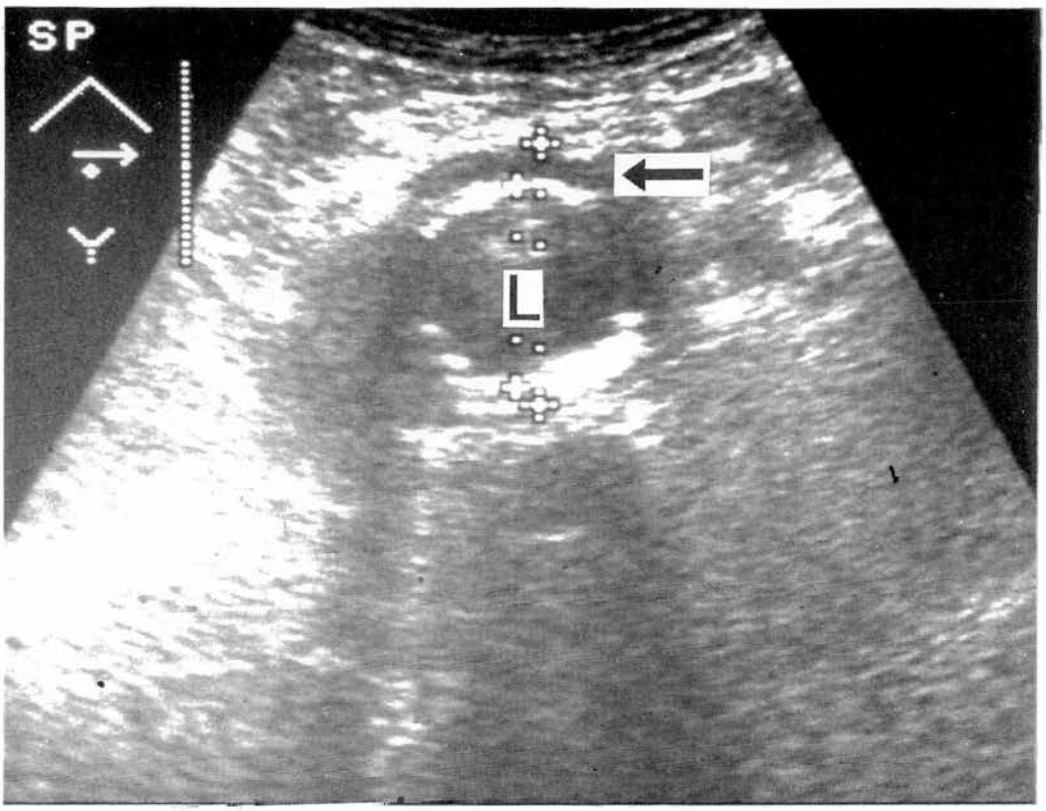
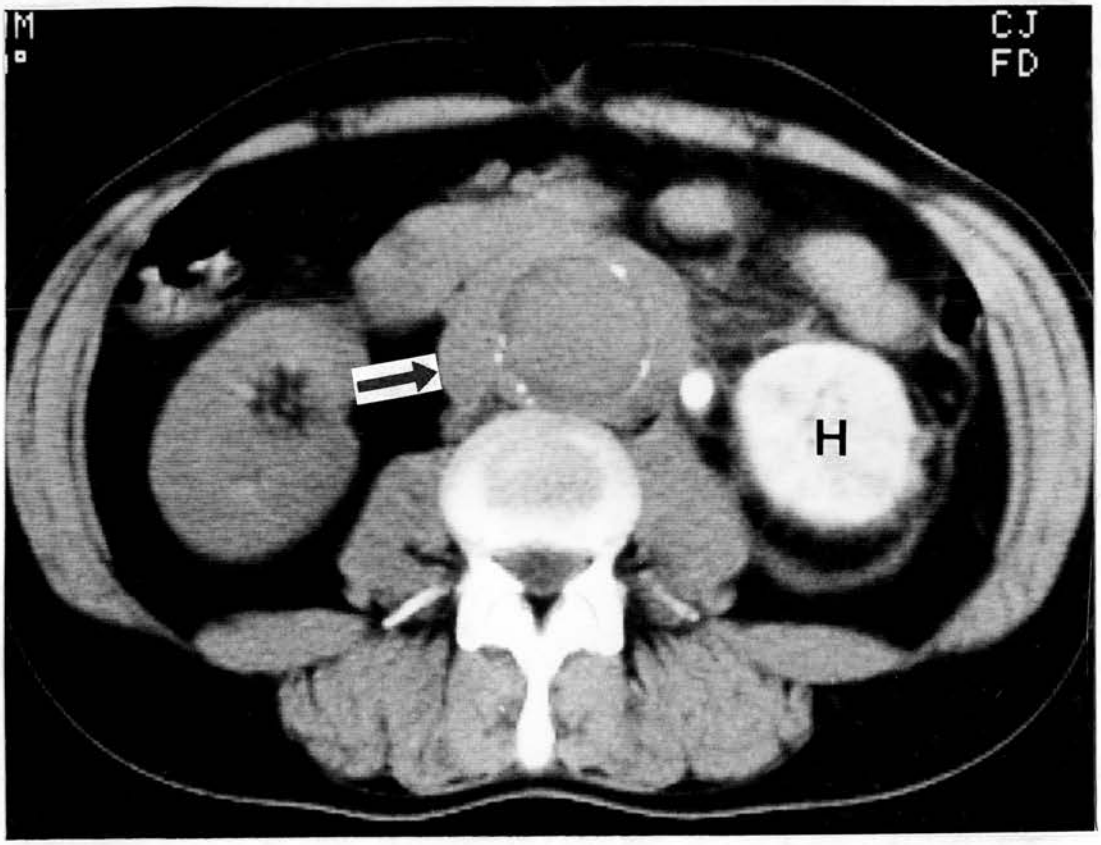


Figure 13.

Contrast-enhanced CT of inflammatory aneurysm. The thick contrast enhancing wall (arrow) is seen surrounding calcification in the aortic media. The patient also has a large left hydronephrosis (H) secondary to ureteric obstruction from inflammatory fibrosis.



MAGNETIC RESONANCE IMAGING - ABILITIES AND CURRENT USE.

Magnetic Resonance Imaging (M.R.I.) is an entirely non-invasive investigation which produces images in a unique way. The tissue to be examined is stimulated to emit a radiofrequency signal by the induction of the phenomenon of nuclear resonance. Images are produced by integration and localisation of this emitted signal. M.R.I. images therefore do not represent attenuation of an external beam, as in conventional x-ray, or reflection, as in ultrasound.

M.R.I. can provide images of any part of the body in virtually any plane with few limitations. Resolution is as good as C.T., and differentiation of soft tissue planes considerably better. No intravenous contrast is required for vascular imaging.[245,246]

Because of the high contrast between fast flowing blood and surrounding tissues, the aortic lumen, wall and branch vessels are all well defined.[247] The technique is capable of displaying saggital and coronal images in addition to transverse, and can therefore delineate vascular anatomy along the long axis of the vessel.

The role of M.R.I. in aortic imaging has been investigated since the early 1980s. As with any new diagnostic technique, considerable study is needed before its place in any given area can be defined.

The only truly non-invasive radiological technique for the diagnosis and monitoring of aneurysm disease until now has been ultrasound. In the thoracic cavity, these functions have been carried out by C.T. and arteriography, both of which require intravenous injection of contrast material.

Because of its non-invasive nature and unique capabilities M.R.I. has established a place in the investigation and monitoring of thoracic aneurysm disease.

Figures 14a-c show MRI images in transverse, coronal and saggital plane.

Figure 14a.

Abdominal MRI image in the transverse plane. The non-inflammatory abdominal aortic aneurysm is indicated by an arrow.

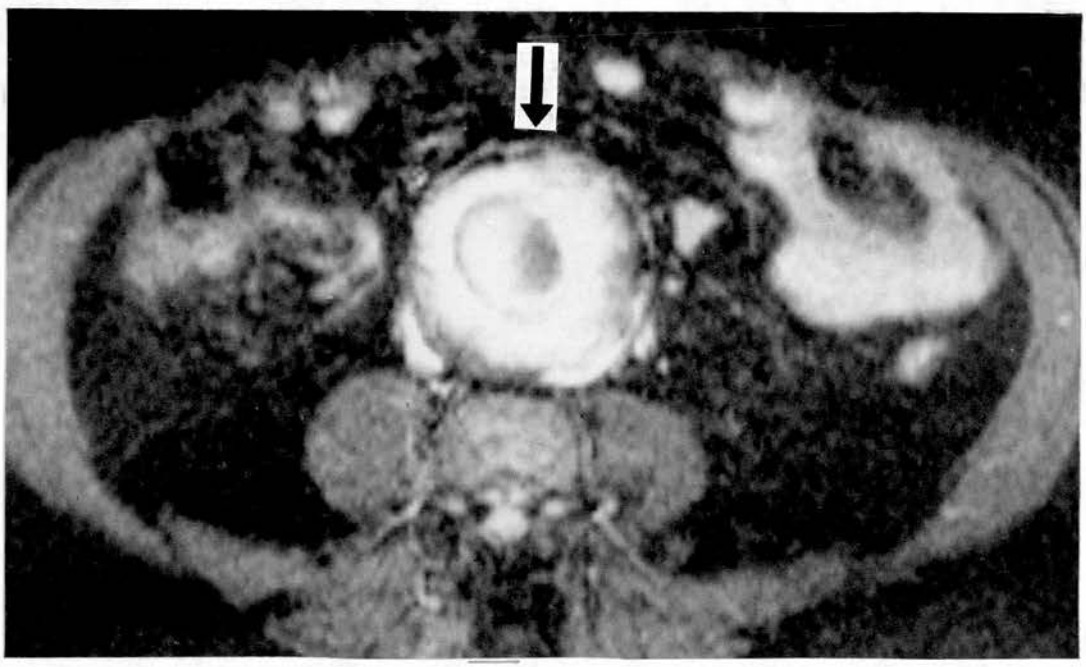


Figure 14b.

Saggital MRI scans of a patient with an abdominal aortic aneurysm (arrows).

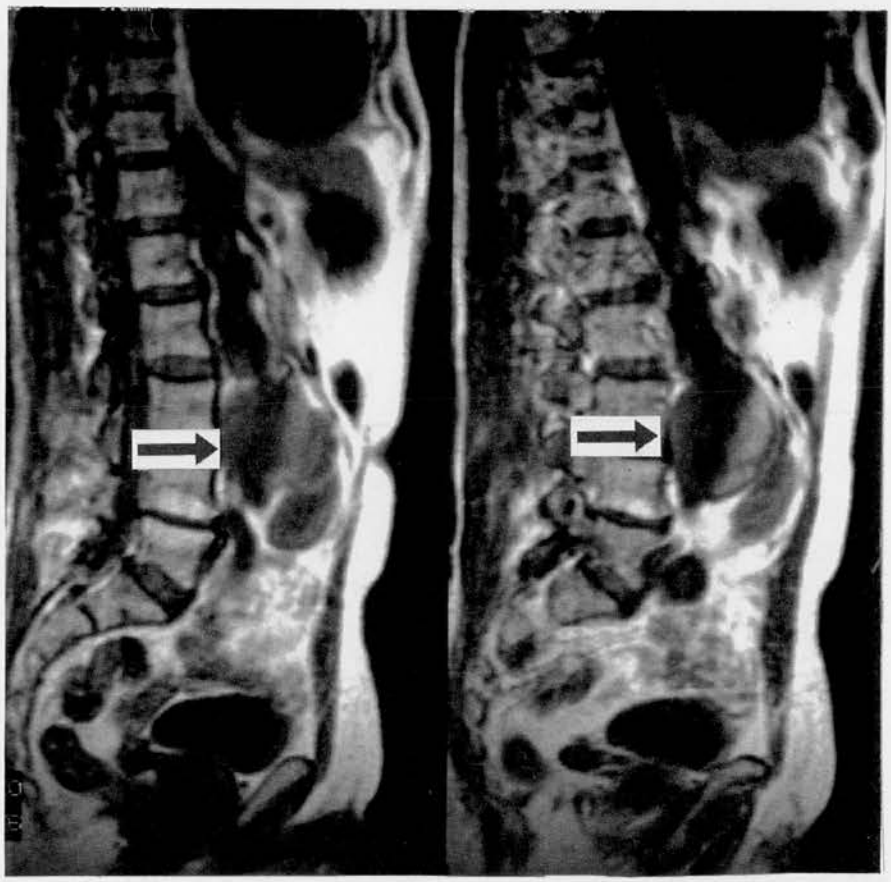


Figure 14c.

Coronal abdominal MRI scan.

M.R.I. has been shown to be reliable in the diagnosis of both aneurysmal and dissecting disease of the thoracic aorta. Entry/re-entry points and true and false lumena of dissection, thrombus adherence and branch involvement can all be demonstrated.[247,248] Akins has suggested that M.R.I. can provide more clinically relevant information than arteriography in cases of thoracic aortic dissection,[249] and both Amparo[250] and Geisinger [251] suggest that M.R.I. is superior to CT in this context.

The series reported by Dinsmore confirms that M.R.I. findings on morphology, location and extent of thoracic aortic aneurysms agree completely with other radiological modalities, and give more information regarding the aneurysm wall.[252]

Although C.T. and angiography will continue to be the most frequently used investigations for the assessment of thoracic aortic disease for some time, imaging using M.R.I. carries many advantages, and may be looked upon in the future as the diagnostic investigation of first choice in thoracic aortic disease.

The role of M.R.I. in the investigation of abdominal aortic aneurysm disease is more difficult to define.

Studies comparing imaging techniques in abdominal aneurysm disease find that M.R.I. compares favourably with both ultrasound and C.T., and Amparo is of the opinion that all the necessary information for aortic surgery can be provided by M.R.I.[253]

Despite this, it is unlikely that M.R.I. will become established as a routine investigation in abdominal aneurysm, at least in the short term, because of cost and availability. Ultrasound remains the ideal non-invasive modality for making the initial diagnosis of AAA and for subsequent monitoring. Necessary information not provided by ultrasound can be gained from

conventional C.T. or arteriography.

There is, however, one aspect of the use of M.R.I. in the diagnosis of abdominal aortic aneurysm which has not been investigated, and in which its particular strengths may prove useful. In inflammatory aneurysms of the abdominal aorta fibrous tissue is prominent in the thickened aortic wall and retroperitoneum. It has been suggested that the ability of M.R.I. to distinguish soft tissue planes is superior to C.T., and it is this ability which requires to be assessed in the diagnosis of inflammatory aneurysms.

MAGNETIC RESONANCE IMAGING - TECHNICAL ASPECTS

While no attempt will be made to provide a detailed technical account of the principles of M.R.I., a brief description of the mechanisms involved is presented.

Basic Principles

Certain atomic nuclei that have an odd number of protons or neutrons possess a characteristic known as 'spin'. Since the nucleus is positively charged, it generates a magnetic field, and behaves like a small bar magnet. The most important nucleus of this type is the proton, a hydrogen nucleus, because of its abundance in tissues.

When tissues are placed in a static external magnetic field, protons align along the long axis of that field, according again to their analogy with bar magnets. In this state, the tissue is said to be 'magnetised'. Full magnetisation, i.e. the time taken to assume a new, aligned equilibrium, takes place exponentially over a finite period. The time taken to reach 63% of full magnetisation is called T_1. At equilibrium, protons may assume one of two energy states, high or low. In normal circumstances, there is a slight excess of low-energy protons.

If a radiofrequency (r.f.) pulse of energy is applied to fully magnetised tissue, some low energy protons are propelled into the high-energy state, where they remain until cessation of the r.f. pulse. When the r.f. pulse stops, these high energy protons 'relax' back into the low-energy state, emitting

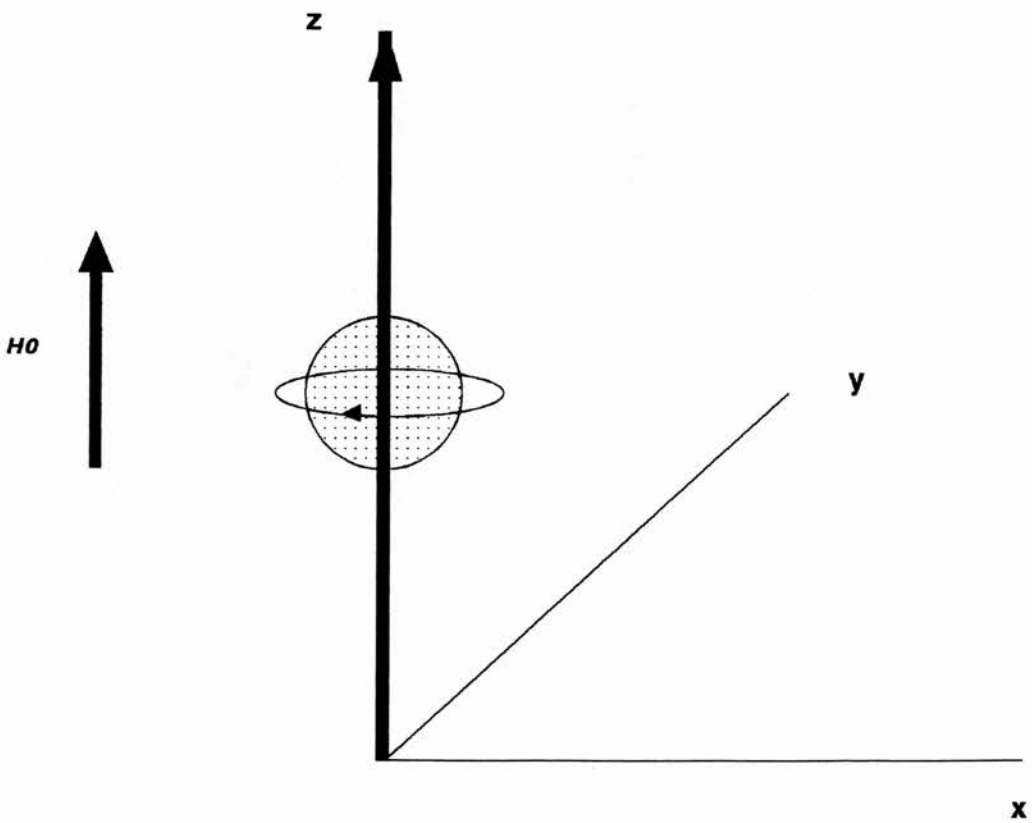
a pulse of r.f. energy at the same frequency as the 'exciting' pulse.

The strength of this emitted energy decays exponentially with a time constant T_2. It is the amplification, localisation and processing of this emitted r.f. energy pulse which is the basis of N.M.R. imaging.

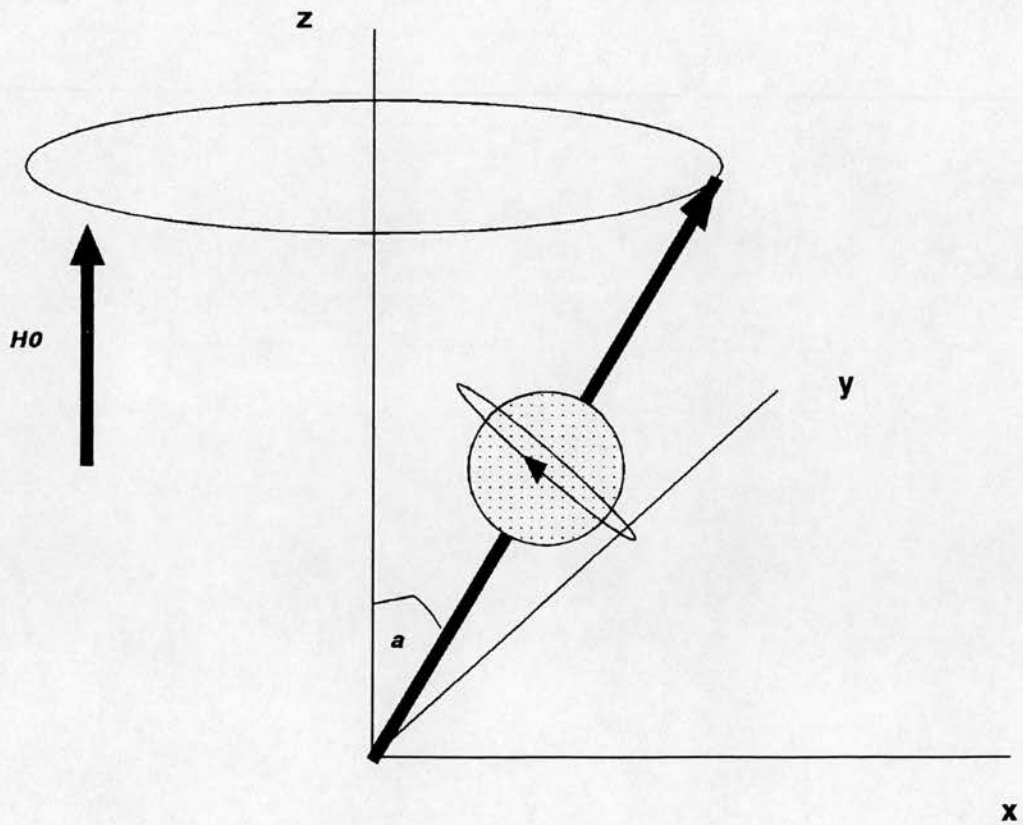
The frequency of the radiofrequency pulse which is required to make a nucleus resonate is dependant on the type of nucleus and the strength of the static magnetic field. In clinical M.R.I., the nucleus examined is always the hydrogen nucleus, a proton, and so the resonating frequency (the **Larmor Frequency**) varies only with the static field strength.

Figure 15 shows, in diagrammatic form, the phenomena described above.

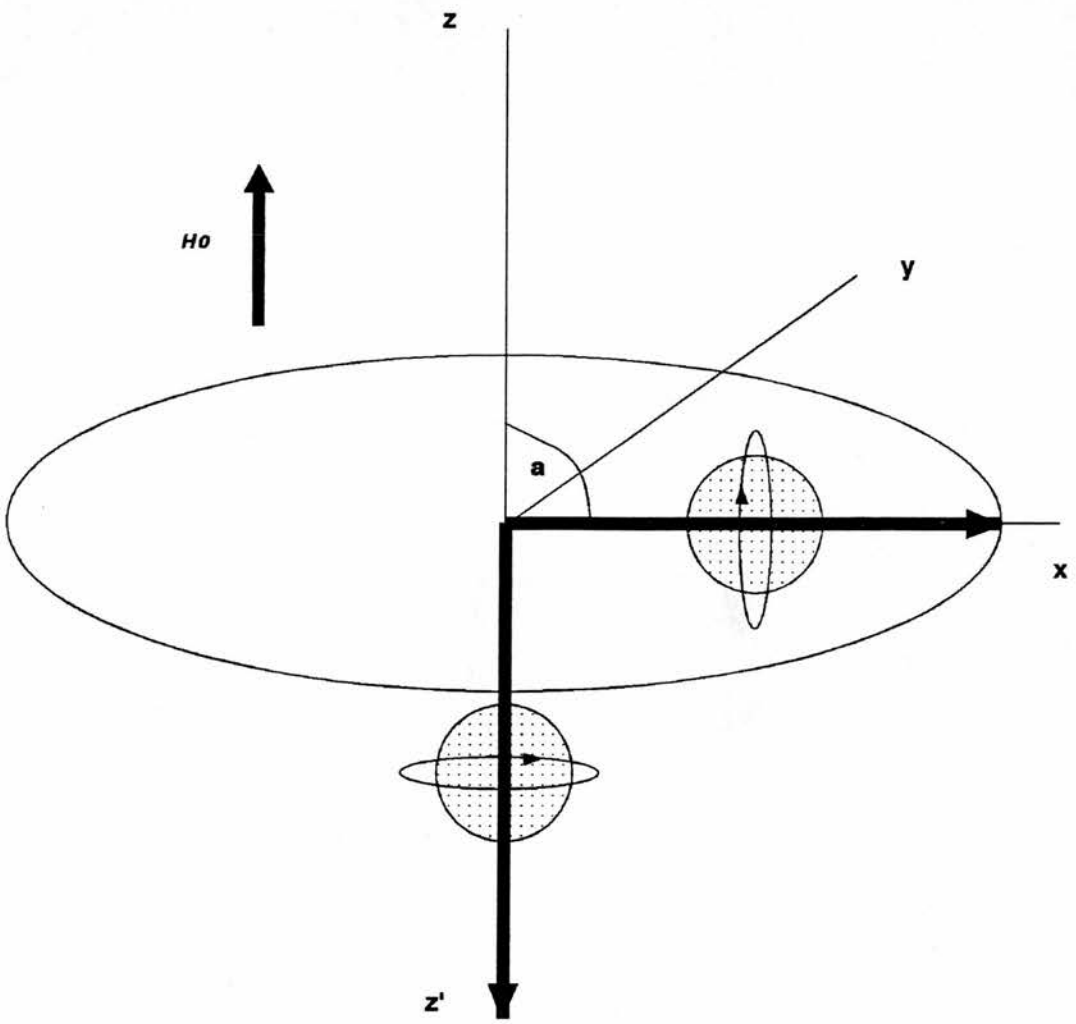
15a. The nucleus is rotating about an axis aligned along the static external magnetic field. H_o indicates the direction of the static magnetic field.



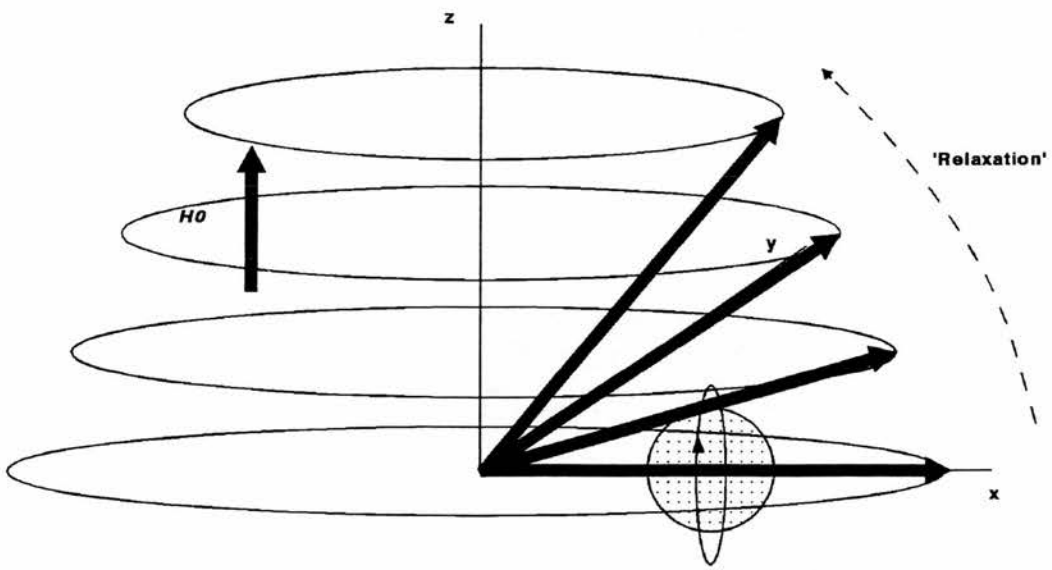
15b. When a radiofrequency magnetic field is applied to this system, aligned in the horizontal plane, the nucleus moves out of the vertical, and wobbles about the vertical axis with a circular path. This wobble is called **precession**, and takes place only when the horizontally applied magnetic field is at a frequency determined by the type of nucleus and the static external field. The frequency required to produce this resonation is the **Larmor Frequency**. The amount of tilt produced in the nucleus (angle α) depends on the strength and duration of the radiofrequency pulse.



15c. The radiofrequency pulse may be sufficient to tilt the axis of nuclear rotation 90°, or 180° from its original vertical. It can be seen that following a 90° tilt, the magnetic moment is maximal in the horizontal plane, whereas following a 180° tilt, the moment is about the original axis, but reversed.



15d. When the radiofrequency pulse is removed, the nuclei begin to relax back to their original position. Immediately after cessation of the r.f. pulse, the nuclei continue to precess around the vertical axis while relaxing. During this time, they are moving around the vertical in a large perpendicular static magnetic field, and so emit a radiofrequency signal of their own, once again at the characteristic Larmor Frequency. It is this signal which is detected, and forms the basis for image production.



Immediately following a 90^0 r.f.pulse, the vertical component of nuclear magnetisation falls to zero. The return to vertical equilibrium is exponential, with a decay constant referred to as the T_1 relaxation time. This time constant is dependant on the transfer of energy from precessing nuclei to other, non-precessing nuclei in the material.

The T_2 relaxation time is the time constant describing the exponential decay of the emitted signal, following cessation of the r.f. pulse. This time constant depends upon loss of phase coherence of precessing nuclei due to interactions between adjacent precessing nuclei.

The biological nature of the T_1 and T_2 time constants are poorly understood, but are considered to be related to the proportion of 'free' tissue water.

In any tissue, some water exists bound to proteins, D.N.A. and other large macromolecules, whilst some water is free. The T_1 of pure water is in the order of 3 seconds: The T_1 of tissue is much lower than this, and depends upon the proportion of water which is 'free'.

T_1 times vary markedly with the resonant frequency of the tissue being examined. As resonant frequency increases with magnetic field strength, so T_1 increases.

Variation in T_2 also occurs with differences in tissue free water, but the variation with changes in magnetic field strength are less marked.

Every tissue in the body can be characterised by its unique value for the time constants T_1 and T_2. Both of these values are determined by characteristics of the tissue at molecular level. Of particular relevance to imaging of the diseased aorta are the T_1 values of fat and water. Medium sized molecules, such as cholesterol have an inherently short T_1 time. Long chain

fatty acids, on the other hand, have a short T_1 time because of rotation about the terminal carbon-carbon bond. The small, rapidly moving molecules of water have a long T_1 time, amounting to some 3 seconds for pure water.

Pulse Sequences

The M.R.I. signal which is recovered from any sample is a function of the way in which the nuclei have been perturbed from their equilibrium state in the static magnetic field. Differences in the timing and duration of radiofrequency pulses will produce differing degrees of nuclear movement and therefore differing M.R.I. signals.

Saturation Recovery

This is the simplest of M.R.I. pulse sequences. In this sequence, repeated pulses are applied, each sufficient to 'flip' the sample nuclei 90^0. The time between pulses, (the repetition time, or TR) is made longer than the T_1 of the sample. As a result the nuclei have time to return to equilibrium between each pulse, and the signal is proportional to the number of nuclei (protons) in the sample. The M.R.I. signal contains no information about the T_1 or T_2 of the sample.

If however, the TR is shorter, for instance equal to the T_1 of the sample, the nuclei do not have time to return to eqilibrium between pulses, and the M.R.I. signal is smaller in amplitude. As the speed with which the sample nuclei regain equilibrium depends on its T_1, the signal, despite being smaller, will contain information about the T_1 of the sample.

Inversion Recovery

This sequence is similar to Saturation Recovery except that the pulses are paired, two pulses being applied every TR. As for Saturation Recovery, the nuclei are not permitted time after the first pulse to return to equilibrium before the second pulse is applied.

The initial pulse in inversion recovery rotates the sample nuclei through a complete 180°, the magnetic moment being rendered large and negative. After the 180° pulse, there is a time delay (Tau) while the nuclei tend to return to equilibrium. Following this delay, and before equilibrium is achieved, a further 90° pulse is applied. This produces an M.R.I. signal with more T_1 information than on simple saturation recovery sequences.

A more detailed description of the physical principles of M.R.I., and other pulse sequences can be found in the excellent review by Bailes and Bryant.[254]

In selecting the pulse sequences to be used in investigation of inflammatory aneurysms, a number of factors were considered.

With the aim of defining the thick wall of inflammatory aneurysms, it was felt that T_1 differences would be relevant. Fibrous tissue has a high water content when compared to the low water / high fat content of atheroma. The pulse sequence most able to highlight T_1 contrasts is inversion recovery.

Disadvantages of M.R.I.

The relatively long scan times necessary for the acquisition of very high resolution images in M.R.I. make it prone to degradation by movement artifact. The intensity and linearity of the magnetic field needed in M.R.I. scanners require patients to be placed in a long tunnel, the walls of which are only a few inches away from their face. Some claustrophobic patients find this unacceptable, and are unable to commence or complete their scan. The enclosed nature of the M.R.I. apparatus also renders M.R.I. less applicable to the investigation of restless or unco-operative patients. The proximity to a large and powerful magnet excludes the scanning of patients requiring ventilators or other extensive monitoring or support equipment which is composed of metallic parts.

The hazards of M.R.I. are produced by the three types of magnetic field used in examination: (a) A strong, static magnetic field. (b) Small, rapidly changing fields. (c) Radiofrequency fields.

As blood flows at right angles to a strong static magnetic field, small electrical charges are set up. This has been observed to result in asymptomatic and harmless E.C.G. changes in some patients.[255] In patients who have recently undergone clipping of cerebral aneurysms, the torque force produced by a strong magnetic field on a high-nickel aneurysm clip may be enough to avulse the clip from the vessel.[256] By the same token, free ferromagnetic material in the proximity of a strong static field can be accelerated to lethal velocity, and is banned from M.R.I. suites.

Small rapidly changing magnetic fields not only may induce current in the leads of cardiac pacemakers, but may change their pacing mode.[257] For this reason, patients with pacemakers should not be scanned. Currents induced by these rapidly changing fields may heat metallic prostheses, and may also lead to tissue effects such as benign visual flashes, muscle contraction and even ventricular fibrillation.

The only potential health hazard posed by radiofrequency magnetic fields are those of prosthesis and tissue heating. This has been shown to be insignificant in clinical use.[255,258,259]

Because of the potential hazards of static and oscillating magnetic fields, restrictions upon strength, rate of field change and radiofrequency exposure are enforced in clinical practice.[255]

SUMMARY

All the information necessary for the surgical repair of abdominal aortic aneurysms is available from ultrasound, angiography, computerised tomography or a combination of these techniques.

M.R.I. allows imaging in all 3 axes, and produces excellent soft tissue definition without the use of contrast material and without having to move the patient. It is likely that M.R.I. alone can provide all relevant information for abdominal aneurysm surgery non-invasively, and give additional information about the peri-aortic soft tissues. The enhanced ability of M.R.I. to display peri-aortic soft tissues was the reason for this study of inflammatory aortic aneurysms.

CHAPTER 5.

BIOCHEMICAL INVESTIGATION OF AORTIC ANEURYSMS - THE ACUTE PHASE PROTEIN RESPONSE

INTRODUCTION.

The interest which has been aroused over recent years in the biochemical changes in aortic aneurysm disease has been reviewed in the preceding chapter concerned with theories of ætiology.

In this chapter, the acute phase response is outlined, and the participating proteins reviewed. The rationale for assay of the acute phase proteins is discussed, as it is for the other biochemical assays which form part of this thesis.

The acute phase protein response

The so-called acute phase reaction comprises a general and nonspecific response to most forms of infective and non-infective inflammatory processes, cell or tissue necrosis, and malignant neoplasia, in which there is a rise in the plasma concentration of certain proteins. The proteins in question are mainly glycoproteins synthesised in the liver, and the available evidence suggests that they act as mediators in the inflammatory process.[260]

Elevation of acute phase proteins occurs in any situation in which there is tissue damage, and is often a more sensitive indicator than ESR, plasma viscosity of leukocytosis. The peak of the acute phase protein rise occurs at about the third day post-injury. In general, the levels of all the acute phase proteins rise in parallel in any given situation, but in some cases certain members of the group are more useful. Cæruloplasmin for example reflects the degree of disease activity in Hodgkins disease, whereas other indices may be normal.[261]

It is likely that products of tissue damage and cell lysis initiate synthesis of acute phase proteins. Such products probably include lysosyme, prostaglandins and interleukin 1, a product of macrophages.

The assay of acute phase proteins in patients with abdominal aortic aneurysms was first carried out by Powell et al. in 1987. They found an increase in levels of alpha-1-antitrypsin, C-reactive protein and cæruloplasmin in patients with aortic aneurysms compared to patients with stenosing aortic disease.[262] As indicated in their paper, this suggests the presence of some degree of inflammation or tissue destruction in all aneurysms.

Proteins involved in the Acute Phase Response

C-reactive Protein (CRP).

C-Reactive protein is a large complex glycoprotein normally present in plasma with a concentration of approximately 6 mg/ml.[261] In evolutionary terms, CRP is one of the most primitive acute phase proteins. An almost identical protein is found in the Horseshoe Crab, indicating the presence of such a protein for 400 million years.

Levels of CRP rise substantially within hours of the onset of inflammation or tissue damage, and it is widely used in this context in clinical practice. Little is known of the function of CRP, but it is thought to act as an non-specific opsonin.[263] C.R.P. binds strongly to many of the polysaccharides commonly found in bacterial cell walls. Once bound, it becomes an extremely potent activator of the classical complement pathway and can therefore initiate cell damaging and inflammatory reactions. Because of its large glycoprotein structure, it is especially effective for capsulated micro-organisms such as pneumococcus. It is also likely that CRP plays a part in platelet

activation and aggregation in the presence of inflammation.

Cæruloplasmin

Cæruloplasmin is synthesised in the liver, and binds approximately 95% of plasma copper. The role of this binding is obscure, as cæruloplasmin does not appear to act as a physiological transport protein for copper. It is a slow reacting acute phase protein, of particular use as an indicator of disease activity in Hodgkins disease, but rises are associated with all inflammatory processes.[261] Cæruloplasmin has been shown to possess anti-oxidant properties, inhibiting peroxidation by both iron and vitamin C. It is also likely that cæruloplasmin inactivates free oxygen radicals produced by phagocytosis. These protective characteristics may justify its acute-phase behaviour.

Alpha-1-antitrypsin (a-1-at).

Alpha-1-antitrypsin is a low molecular weight protein which is able to pass freely throughout all body fluids. In human plasma it is normally present in concentrations of approximately 2 g/l, but rises in inflammation with a response time of approximately 24 hours. It is a protease inhibitor which is most active against serine proteases such as elastase and trypsin, but which is also known to bind to and inhibit other proteases such as cathepsins B1 and D, and collagenase, a metalloprotease. Inhibition of proteases corresponds with the formation of a complex between a-1-at and the protease.

It seems probable that a-1-at scavenges proteases in tissue fluids and then returns to the plasma space from which the proteases are eliminated. Reduction in the activity of a-1-at is known to be associated with a series of disease states as a result of unopposed protease activity, which are outlined below.

Alpha-1-antitrypsin shows marked genetic polymorphism, and some 30 allotypes have been isolated.[261] The various phenotypes can be separated by electrophoretic techniques, most commonly isoelectric focussing. Differences in the allotypes may depend upon the post-transcriptional carbohydrate heterogeneity superimposed upon charge differences resulting from inherited substitutions in the polypeptide chain. The clinical interest surrounding a-1-at has centred on the association of certain allotypes with disease states.

The genes and corresponding phenotypic variants of a-1-at are assigned names prefixed with Pi (**P**rotease **i**nhibitor). The most clinically relevant genotypes are PiM, PiW, PiS, PiP, PiZ and Pi null. Inherited in Mendelian fashion, these genes give rise to phenotypic variants of a-1-at including PiMM, PiMZ and PiMS. The population incidence and relevant activity of several example phenotypes of a-1-at are shown in Fig 16.

Figure 16.

Phenotype	Incidence	Activity
PiMM	80%	100%
PiMS	9%	80%
PiMZ	3%	57.5%
PiZZ	0.03%	15%

The reasons for variation in hepatic secretion of a-1-at depending upon phenotype probably relate to structural differences between phenotypes. The ability of hepatocytes to secrete a-1-at varies with phenotype (and therefore structure), accounting for differences in plasma levels, and the accumulation of a-1-at within the hepatocytes of some PiZZ infants (see below).

Clinical disease is associated with the phenotypes PiZZ and PiSZ, both of which are poorly secreted by the liver, leading to considerably reduced plasma levels of a-1-at. In the presence of low levels of a-1-at, protease activity is poorly opposed, leading to increased proteolysis.

In the lung, any stimulus associated with phagocytosis results in a local increase in leukocyte proteases. In the presence of adequate levels of antiproteases such as a-1-at, these proteases are quickly inhibited, and their potential for damaging other tissues limited. When there are low levels of a-1-at, unopposed proteolysis leads to damage to elastin, and results in emphysema.

About 20% of infants with the PiZZ phenotype develop neonatal hepatitis. The livers of all the affected individuals show PAS positive granules, found on immunofluorescence to be composed of a-1-at. It is this finding which supports the suggestion that decreased production is secondary to failure of hepatic secretion of a defective protein.

A-1-at is an important inhibitor of elastase. Serum activity of a-1-at is genetically determined, and depends on phenotype. Certain phenotypes which result in markedly reduced activity have been associated with clinical disease secondary to unopposed action of protease.

Alpha-2-macroglobulin (a-2-mg).

Alpha-2-macroglobulin is also an important antiprotease.[264] As with a-1-at, protease inhibition is the result of binding of the a-2-mg to the protease to form an inactive complex. A-2-mg is a large protein, entirely confined to the vascular compartment. Its half life, when complexed with proteases is in the order of 10 minutes. A-2-mg probably acts as a secondary carrier and inhibitor of protease molecules following their transfer from a-1-at. Its role as an acute phase protein is dubious, but its interaction with a-1-at, and its function as a plasma antiprotease justify its measurement in investigation of inflammatory aneurysm for the same reasons as for a-1-at.

Use of Acute Phase Protein Assay in Aneurysm disease.

From the evidence presented in chapters 2 and 3, aortic aneurysms have been associated with decreased elastin in their wall together with increased elastase activity in both wall and peripheral blood. Decreased levels of antiprotease activity capable of inhibiting elastase have also been found in patients with aneurysm disease. In inflammatory aneurysms there is an active inflammatory cell infiltrate and severe elastin destruction accompanied by increased levels of neutrophil elastase.

The measurement of the acute phase proteins, and plasma levels, activity and phenotype of alpha-1-antitrypsin may therefore be of use in the investigation of inflammatory aneurysm of the abdominal aorta. Raised levels of acute phase proteins may indicate an inflammatory response and suggest metabolic activity within the aneurysm wall.

The detection of low antiprotease activity levels or phenotypes of a-1-at associated with poor production might indicate deficiencies in the antiprotease system and an increase in unopposed proteolysis in the aortic wall. Such defects, resulting in a net increase in proteolytic activity, would be compatible with the fierce and active elastolysis seen in inflammatory aneurysms.

Elastase Complex Assay

Assay of the complex of a-1-at and neutrophil elastase has been described as a useful clinical indicator in other inflammatory diseases.[265,266] As this elastase has been associated with inflammatory aneurysms, its measurement in peripheral blood may be of use in determining the pathogenesis of inflammatory aneurysm disease, and also in its diagnosis.

Any alteration in the protease/antiprotease balance which may be detected could be implicated in the elastolysis seen in inflammatory aneurysms. The evidence that this is the case would be strengthened by the biochemical confirmation that increased elastin breakdown is taking place.

Summary

The acute phase proteins are a group of glycoproteins the levels of which rise non-specifically in inflammation. They may therefore respond in this capacity to the presence of an inflammatory aneurysm. Of particular interest are alpha-1-antitrypsin and alpha-2-macroglobulin. In addition to their participation in the acute phase response, they have an important role in the anti-protease system.

Inflammatory aneurysms are particularly associated with marked elastin destruction in the tunica media of the aorta by elastase. Any local or systemic alteration in antiprotease activity can be expected to result in increased, unopposed elastase activity, and may be of relevance in the pathogenesis of inflammatory aneurysms.

The biochemical investigations included in this Thesis are therefore the following:

1. Acute phase protein assays. The proteins assayed will be c-reactive protein, cæruloplasmin, alpha-1-antitrypsin and alpha-2-macroglobulin.

2. Neutrophil elastase. This will be assayed in the peripheral blood in its complexed form with alpha-1-antitrypsin.

3. Electrophoretic phenotyping of alpha-1-antitrypsin.

4. Functional assay of alpha-1-antitrypsin in the peripheral blood (elastase inhibitor assay).

5. Elastin fragment peptide assay in peripheral blood.

CHAPTER 6.

PATIENTS AND METHODS

INTRODUCTION

The following chapter contains details of the patients studied in this work. The techniques used for the collection, storage and retrieval of pertinent data are specified.

Experimental techniques and equipment used in each of the protein assays used are described, as are the parameters used for each radiological investigation.

PATIENTS

Retrospective Patients.

The experience of the Bristol Vascular Studies Unit in aortic aneurysm surgery was reviewed over a 5 year period. Information was taken from the computerised prospective surgical audit system, B.I.P.A.S. Also reviewed were patients case notes, including anaesthetic records and operation notes. For long-term follow-up data, each patients General Practitioner was contacted either by telephone or letter.

In the cases where the diagnosis of inflammatory aortic aneurysm is used, this diagnosis was made on the grounds of inspection at operation, together with histopathological examination using previously published criteria.[1]

47 patients with inflammatory aortic aneurysms are included. In all cases, aneurysmal change was limited to the infrarenal aorta. For comparison, a group of 162 patients with simple, non-inflammatory aneurysms presenting over the same time period are included.

This group does not represent the entire caseload of simple aneurysms in the five year period, but only those in whom aneurysmal change was limited to the infrarenal aorta. It is only in this way that valid comparisons of operative time and blood loss can be made, and an impression gained of the degree to which inflammatory change demands changes in operative technique.

Prospective Patients

All patients presenting for elective aortic surgery were considered for inclusion in the study. Specific exclusion from study was made in the following cases:

(a) Those who refused consent for any reason, or unable to understand the implications of consent.
(b) Those with known malignant disease.
connective tissue disease.
inflammatory bowel disease.

In patients with aorto-iliac occlusive disease, no specific radiological investigations were undertaken for the purposes of the study.

Patients with aneurysms underwent radiological investigation by three modalities: Ultrasound, contrast-enhanced computerised tomography and magnetic resonance imaging. Following diagnosis, each patient was sent the letter in appendix 2 seeking information relevant to M.R.I. investigation. Patients were excluded who had implanted pacemakers likely to be affected by the magnetic fields.

Data Storage and Retrieval

Computerised data handling was used throughout, although written copies of all information were retained.

All patient details were stored in a structured database file using *dBase 3+* software. Numerical data was stored in spreadsheet form using *Lotus 2.1*, which was also used to calculate regression lines for some of the protein assays.

From the spreadsheet, selected data was transferred to *Oxstat* for analysis.

Completion of questionnaire

A questionnaire was completed for each patient at a pre-operative interview, the style of which can be seen in appendix 3. At the same interview, permission was sought from the patient for use of blood and aortic specimens for research purposes. The format, which was verbal consent only, was approved by the Ethical Committee of Bristol & Weston Health Authority.

Details of medical history, drug intake, concomitant disease etc., were confirmed from medical records in all cases.

Collection of Blood Samples

All samples were collected immediately pre-operatively from fasting patients using the Vacutainer system.

Samples for enzyme and acute phase protein assays were then separated by centrifugation within ½ hour of collection at 2000 rpm for 15 minutes. Serum and plasma were kept at -80 degrees centigrade in aliquots of 1 ml until used for analysis.

Appropriate samples were sent to the hospital laboratory for full blood count and plasma viscosity measurement.

Collection of Tissue Samples

Samples of aortic wall were taken from the anterior part of the aneurysm sac opposite the inferior mesenteric artery origin, or at the point of maximum dilation in suprarenal or thoracic aneurysms.

The tissue was cut into small pieces and snap frozen in liquid nitrogen for histological studies. Larger pieces were subjected to MRI examination in the fresh state.

Acute Phase Protein Assays

Principle.

All assays of acute phase proteins were carried out using immunoturbidimetry.

Immunoturbidimetry is an immuno-assay technique which depends upon the optical qualities of an antigen/antibody interaction in solution. Using this technique, a fixed volume of antigen-containing serum is added to a fixed volume of a standard solution of antibody, with antibody excess. As antigen and antibody combine to form aggregates, the optical density of the solution

increases. As there is antibody excess, the rate of the reaction is rapid at first, but plateaus as antigen is consumed in the formation of antigen/antibody aggregates. The maximum rate of change in optical density observed over the reaction period is proportional to the amount of antigen present in the original sample.

Standard absorbance curves are constructed for all assays by first assaying serial dilutions of standard calibrators.

The calibrators contain known concentrations of the protein antigen under assay. Serial dilutions of calibrator are made and assayed using standard antibody. The maximum rate of change of optical density over the reaction period is defined for each dilution. As antigen concentrations in the dilutions are known, the relationship between maximum reaction rate and antigen concentration can be made.

In this way, a standard curve is derived, according to the protocols below, relating maximum rate of change in absorbance to absolute antigen concentration. Antigen concentrations in patient samples can then be assayed.

Patients.

Serum samples from 15 patients with inflammatory aneurysms, 82 with simple aneurysms, and 37 with severe aorto-iliac occlusive disease were assayed for each of the acute phase proteins.

Procedure.

All acute phase protein assays were carried out using the computer orientated random access immunoturbidometer shown below in Figure 17 (COBAS MIRA, F. Hoffman-LaRoche, Basle, Switzerland).

Figure 17.

LaRoche COBAS MIRA analyser used in acute phase protein assays.

Stored serum samples taken from study patients were thawed at room temperature. In all assays, samples were diluted using 0.9% sodium chloride to a dilution of 1:20 - 1:30. 6% Polyethylene glycol was used as an enhancement reagent in all cases.

A list of the reagents used in each of the assays is given below.

1. Serum alpha-1-antitrypsin assay.

Antibody - Atlantic antibodies anti alpha-1-antitrypsin.
Calibrator - Atlantic antibodies Calibrator 3, in 6 serial dilutions.
Controls - Atlantic antibodies Calibrator 1, SPSO1

2. Serum Caeruloplasmin assay.

Antibody - Atlantic antibodies anti caeruloplasmin
Calibrator - Atlantic antibodies Calibrator 1 in 6 serial dilutions.
Controls - Atlantic antibodies Calibrator 3, Behring protein standard plasma.

3. Serum alpha-2-macroglobulin assay.

Antibody - Atlantic Antibodies anti alpha-2-macroglobulin.
Calibrator - Atlantic Antibodies Calibrator 1 in 6 serial dilutions.
Controls - Atlantic Antibodies Calibrator 3, Behring standard human serum.

4. Serum C-reactive protein assay.

Antibody - Atlantic Antibodies anti C-reactive protein.
Calibrator - Atlantic Antibodies Calibrator 7 in 6 serial dilutions in bovine serum albumin.
Controls - Atlantic Antibodies Calibrator 7 prediluted 1:2 and 1:4 with bovine serum albumin to bring into range of standard curve.

[N.B. C-reactive protein is present in serum in very small concentrations. Undiluted serum must therefore be used for assay. When constructing standard curves with calibration standards (in this case Atlantic Antibodies Calibrator 7), it has been found that reaction kinetics of saline-diluted calibrators at high dilutions do not compare with the 'neat' serum samples being analysed. These matrix effects are obviated by using bovine serum albumin as a diluent for standards.]

Standardisation.

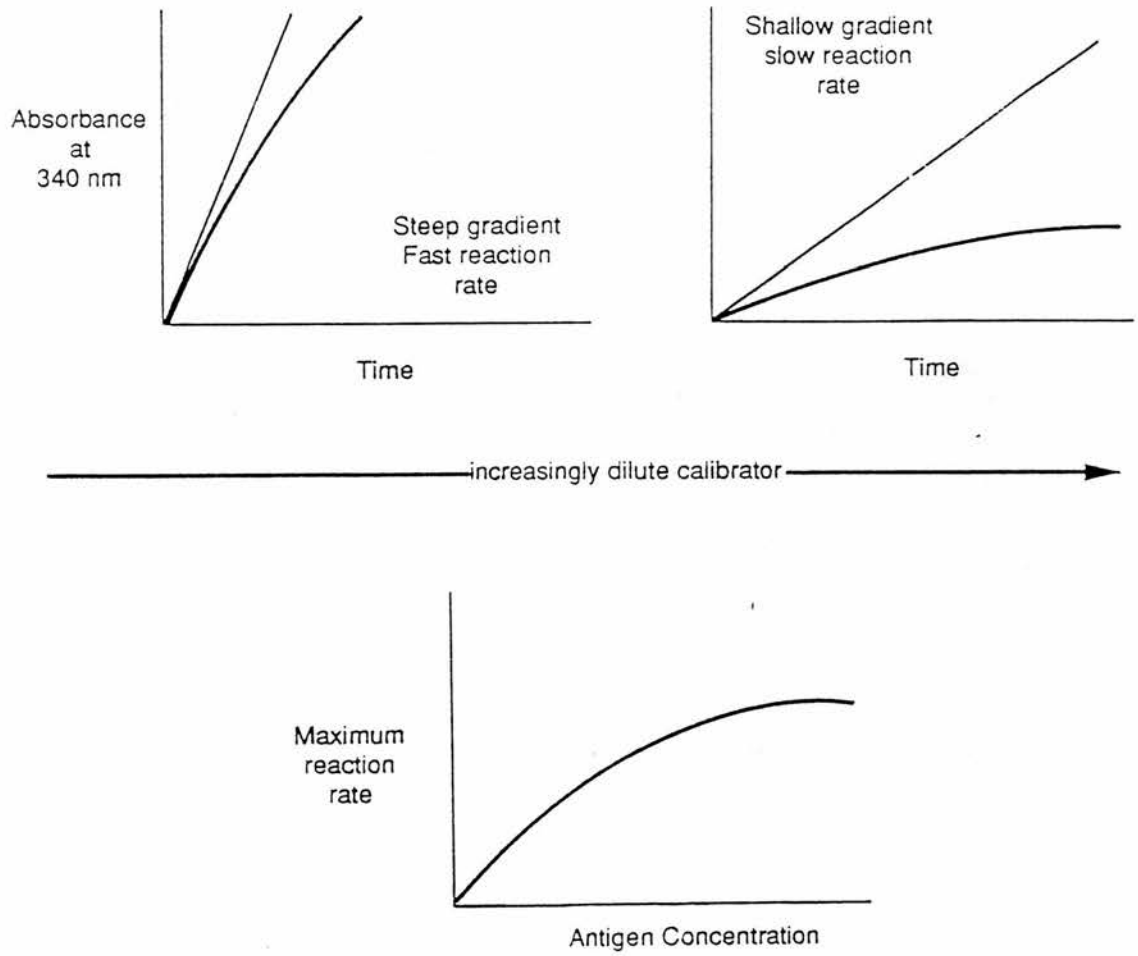
In normal laboratory use, calibration of the the analyser is maintained to give a coefficient of variation for protein assays of 2-3%. The

procedure used to calibrate the COBAS MIRA is illustrated below in Figure 18.

For each assay run, 1 sample in 10 was a control specimen of known antigen content. Their absorbance and concentration was used to validate the standard absorbance curve

Figure 18.

Calibration of the COBAS MIRA, using increasingly dilute solutions of a calibrator of known assay. Reaction rate is plotted, and the maximum rate noted for each concentration. Maximum reaction rate is then plotted for each antigen concentration. This graph is used to calculate antigen concentration of unknown samples using maximum reaction rate obtained at assay.



Results obtained from the acute phase protein assays were stored on computer for later statistical analysis. Both the results and analysis can be found in Chapter 8.

Elastase-Antitrypsin Assays.

Principle.

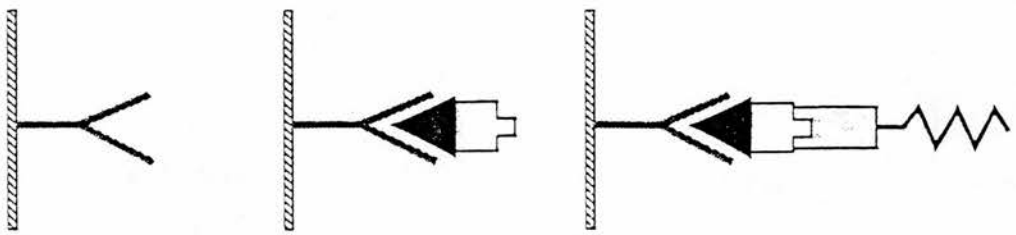
Elastase-Antitrypsin Assays were performed using Enzyme-Linked Immunosorbent Assay, the principle of which is described below.

Plastic tubes coated with sheep antibody to neutrophil elastase are used. A standard volume of serum is added to the tube, and the contained elastase-antitrypsin complex is bound to the tube wall by its elastase moiety. The amount of binding is proportional to the amount of elastase-antitrypsin complex present in the sample. The tube is then washed, and a conjugate of antitrypsin antibody/alkaline phosphatase added. This binds in turn to the antitrypsin moiety of elastase-antitrypsin complex, forming a complex with an enzymatic 'tail' of alkaline phosphatase. After washing, the tube-bound enzyme activity is measured by its action on 4-nitrophenyl phosphate, producing a colour change at 405 nm. The colour change is measured by absorbance spectrophotometry and is proportional to the amount of elastase-antitrypsin complex in the original sample.

The principles of E.L.I.S.A. are illustrated in Figure 19.

Figure 19.

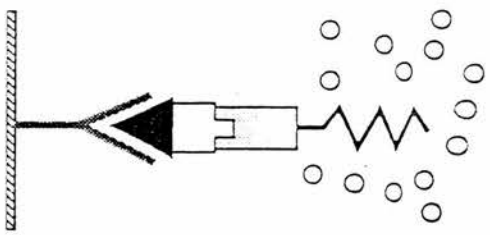
The principle of Enzyme Linked Immunoabsorbent Assay (E.L.I.S.A.)



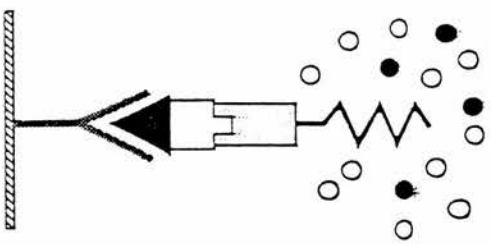
Sheep anti elastase bound to reaction tube wall

Serum added, containing elastase anti trypsin complex. Bound to anti elastase by elastase moiety

Anti antitrypsin / alkaline phosphatase conjugate added binds to antitrypsin part of complex



4 - nitrophenyl phosphate substrate added. Phosphatase enzyme catalyses colour change



Colour change detected by absorbance spectrophotometry at 405 nm

Reaction stopped at one hour

Patients.

Elastase-antitrypsin complex assays were carried out on stored plasma samples from 15 patients with inflammatory aneurysms, 66 with simple aneurysms, and 32 with severe aorto-iliac occlusive disease.

Procedure.

A commercially available assay kit was found to be suitable, available from Merck Immunoassay, kit number 12589 (2h version), for Polymorphonuclear Leukocyte Elastase. The assays were carried out using the kit in a rack of 48 pre-prepared plastic test-tubes. Each kit contained two racks each of 24 test tubes coated as detailed above. This provided for the simultaneous assay of 1 reagent control, 4 assay calibrator standards, 1 control plasma, and 18 patient samples all in duplicate.

The 4 standard calibrator solutions of known elastase/antitrypsin complex content were assayed in order to produce a standard line of absorbance versus elastase-antitrypsin concentration. The accuracy of the line was verified by assay of a control serum, and then used to determine the elastase antitrypsin content of the unknown samples.

A detailed protocol for this assay is found below. A list of reagents for the assay is given, followed by an illustration of reaction tube allocation (Figure 20), and a detailed protocol for the assay.

List of Reagents required for elastase antitrypsin assay

Calibrator standards - X4: Gelatine containing TRIS buffer, stabilizer, PMN Elastase-α1 proteinase inhibitor complex. Each diluted with 5 ml of dilution medium.

Control Plasma - Human plasma of known E-α1AT complex assay. Diluted with 500μl of dilution medium.

Sample Dilution Medium - Phosphate buffer pH 7.52

Wash Solution - Water, detergent. Diluted 1:1 with distilled water.

Substrate Buffer - Diethanolamine pH 9.8 (1 mmol/l), $MgCl_2$(0.5 mmol/l).

Substrate - Tablets containing 20 μmol 4-nitrophenyl phosphate. Each dissolved in 10 ml substrate buffer.

Stop solution - Sodium hydroxide 2 mmol/l

Antibody/enzyme conjugate - Antibodies to α1 proteinase inhibitor from human serum, coupled to alkaline phosphatase. Diluted with reconstituted Antibody-Enzyme conjugate buffer.

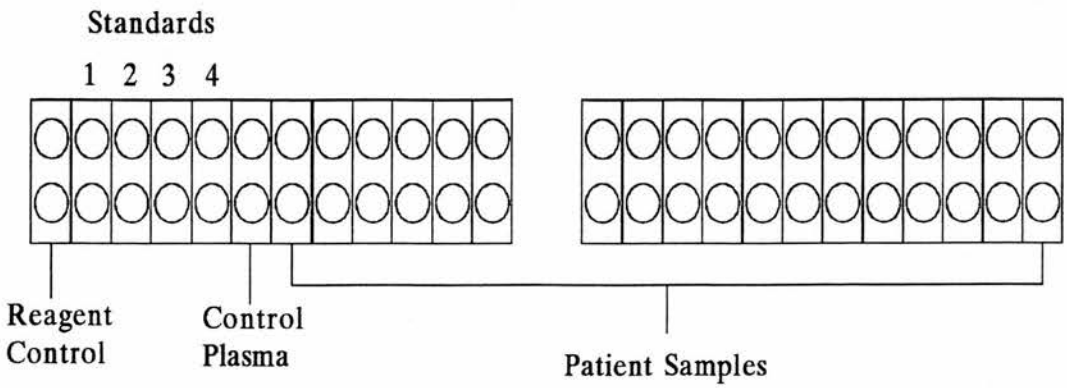
Antibody/enzyme conjugate buffer - TRIS pH 7.5, $MgCl_2$Reconstituted to 25 ml with wash solution.

Antibody coated tubes - polystyrene tubes coated with sheep antibodies to human granulocyte elastase.

Samples of stored plasma from patients were thawed at room temperature and diluted using a mechanical diluter.

Figure 20.

Allocations for reaction tubes in the elastase antitrypsin assay kit. Four standard solutions of antigen are assayed with a control plasma also of known assay. All elastase assays for unknown samples are assayed in duplicate.



All assays carried out in duplicate
Final volume 600 μl.

Table of Procedure for elastase antitrypsin assay.

	TUBE			
	Reagent Control	Calibrators 1 - 4	Control Plasma	Samples
Wash Solution	1000µl	1000µl	1000µl	1000µl
Incubate at at 20-25°C.for 5-20 min then draw off to dryness				
Dilution medium	500 µl			
Calibrators 1-4		500 µl		
Control Plasma			500 µl	
Samples				500 µl
Incubate at 20-25°C. for 60 min, then draw off to dryness				
Wash Solution	1000µl	1000µl	1000µl	1000µl

Draw off to dryness, then repeat wash X 2 with 1000µl aliquots of wash solution. Draw off to dryness.

Antibody/enzyme solution	500 µ	1500 µl	500 µl	500 µl
Incubate for 30 min, then draw off to dryness				
Wash Solution	2000µl	2000µl	2000µl	2000µl

Draw off to dryness, then repeat wash X 2 with 2000µl aliquots of wash solution. Draw off to dryness.

Substrate Solution	500 µl	500 µl	500 µ	1500 µl
Incubate for 30 min protected from light.				
Stop Solution	100 µl	100 µl	100 µl	100 µl

Mix thoroughly. Prepare mixture of 500 µl of substrate solution and 100 µl stop solution, and read absorbances of all tubes against this, at 405 nm.

Absorbances were measured at 405 nm using a Pye Unicam spectrophotometer, and a light path of 1 cm.

Samples were read against the blank prepared above, of substrate and stop solution.

The results of each assay run were stored on *Lotus 123* spreadsheet software. A standard line was plotted using the absorbance results and known concentrations of the four standards. The regression function of Lotus was used to calculate the equation of the best fit straight line for these four points. This equation was used to calculate elastase/antitrypsin concentration from absorbance readings of both unknown samples and the control plasma.

Standardisation of Assay.

Standardisation of this assay was ensured by the assay of four calibration standards and one control plasma sample in each kit. All samples were assayed in duplicate. Kits in which the control plasma value as assayed deviated unacceptably from its known value were rejected, and the results of patient samples from that kit were not used.

The table below shows the details of each assay kit in terms of the known value as given in the kit literature, the value calculated from the assay. The difference between the two values is expressed as a percentage. Asterisks are placed beside kits from which results were disregarded.

2. Control Plasma Values (μg/l)

Kit No.	Known value	Calculated Value	% Difference
*1	125	169	35.2
*2	60	72.24	20.4
*3	60	75.77	26.2
4	135	145.99	8.1
5	135	128.24	5.0
6	60	65.61	9.3
7	60	66.62	11.0
8	60	65.75	9.5
9	60	64.37	7.2
10	60	55.99	6.6
11	60	61.91	3.1
*12	60	45.86	23.5
13	55	52.04	5.3
14	60	56.53	5.7
15	55	59.79	8.7
16	55	61.6	12.0

Kits marked with * were deemed inaccurate, and results not included in study

The results of reproducibility studies, and the results of patient assays can be found in Chapter 8.

Elastase Inhibitor Assay

Principle.

This assay was originally devised in the University of Sheffield Protein Reference Laboratory. A number of changes have been in the procedure with regard to reagent dilution and incubation times, but the technique remains the same.

The principle of the assay depends on the inhibitory power of patients serum on a standard elastase load. Patients serum is added to a fixed amount of porcine elastase, and incubated. During incubation, the standard elastase load is inhibited to a degree proportional to the amount of anti-elastase activity in the patients serum. The resultant serum/elastase mixture is then used to digest a fixed amount of elastase substrate. The more substrate digested the less elastase inhibitor present in the patients serum.

Digestion of the substrate produces a colour change detectable by absorbance spectrophotometry at 405 nm which is proportional to the amount of elastase inhibitor in the patients serum.

Patients.

This assay was carried out on the stored serum of 15 patients with inflammatory aneurysms, 67 with simple aneurysms, and 33 with aorto-iliac occlusive disease.

Procedure.

The assay was carried out on 96 well microtitre plates. On each plate, five wells were used to assay known dilutions of stock elastase solution. Three calibrator samples (see below) were also included on each 96 well plate. Using this format, 40 unknown samples may be assayed on each 96 well plate.

An account is given below of the preparation of the reagents required for this assay. It is followed by a detailed protocol including an illustration of reaction well allocation of the 96 well microtitre plates. (Figure 21)

Reagents for Elastase Inhibitor Assay.

TRIS buffered saline: 0.9% sodium chloride, 0.1M TRIS, pH 8.5.

Elastase: Type 4 chromatographically purified porcine pancreatic elastase. (Sigma Chemicals).

Elastase Substrate: N-succinyl-ala-ala-ala-p-nitroanilide (S.A.N.A.). (Sigma Chemicals).

Preparation of Elastase.

Purified porcine pancreatic elastase (Sigma) 70 iu/mg made up in TRIS buffer to 0.02 mg/ml.

Preparation of S.A.N.A. Elastase Substrate

N-succinyl-ala-ala-ala-p-nitroanilide (Sigma) made up to 1 mg/ml in TRIS buffer.

Dilution of Samples

Samples taken into E.D.T.A. from fasting patients. Stored at -80^{O} until used. Thawed to room temperature. Diluted 1:30 with TRIS buffer using mechanical diluter.

Preparation of Elastase Dilutions.

Stock elastase solution of 0.02 mg/ml was diluted in TRIS buffer to produce 25%, 50%, and 75% dilutions. TRIS only and elastase solution 0.02 mg/ml only were used for 0% and 100% elastase activity calibrators. These dilutions therefore represented 0% - 100% elastase activity, and were used in calibration wells 1-5 (see below).

Wells in the microtitre plate were assigned numbers from 1-96 running vertically from top left.

Well 1: Zero control. 100μl TRIS.

Wells 2-5: Elastase dilutions 25% - 100% 50μl, TRIS 50μl.

Wells 6-8: Standard sera 50μl, elastase 50μl.

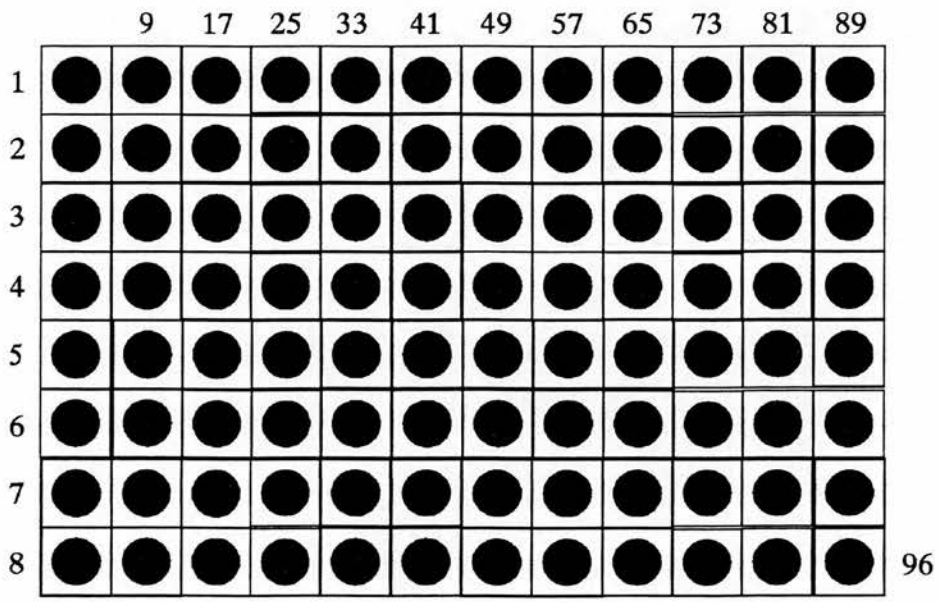
Wells 14-16: Standard sera 50μl, TRIS 50μl. (Blanks for standard sera.)

Wells 17-24, 33-40, 49-56, 65-72, 81-88: Test wells. Diluted serum 50μl, elastase 50μl.

Wells 25-32, 41-48, 57-64, 73-80, 89-96: Blank wells. Diluted serum 50μl, TRIS 50μl.

Figure 21.

Well numbering for elastase inhibitor assay. The wells are numbered from 1-96. Contents of each well are given above.

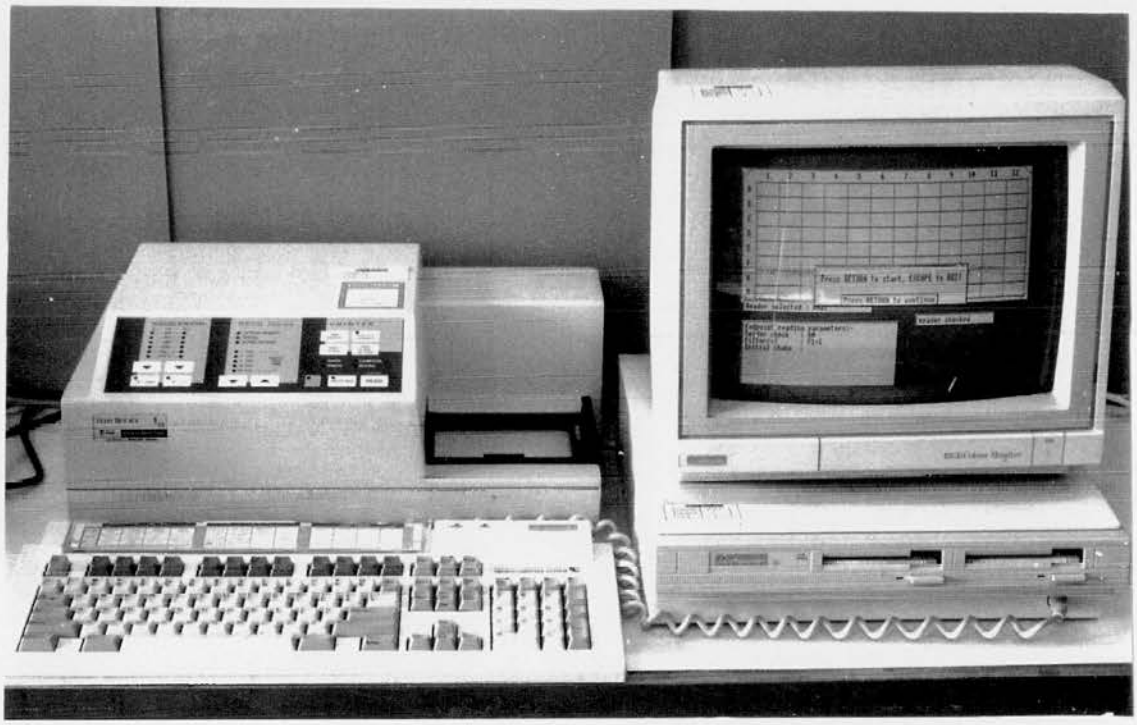


The plate was then incubated for 30 minutes at 37°C, following which, 100μl of SANA was added to each well. The plate was then incubated at room temperature in the dark for a further 1 hour. Absorbance was read at 405 nm.

All results were obtained by reading the absorbance of the samples on a Novoclone microplate spectrophotometer controlled by dedicated software running on a BBC Archimedes 310 computer. (Figure 22)

Figure 22.

The Spectrophotometer and Archimedes computer combination used in the assay of antiprotease activity



A standard curve was drawn using results of absorbance from wells 1-5, and their known percentage of elastase activity. The absorbance of unknown samples were then related to percentage elastase activity using this standard curve. The three known calibrator samples were used to validate the results obtained by each plate of assays. Only if good separation was obtained, was the curve used to read unknown samples.

Standardisation of assay.

The method given to us differed from that detailed above, as originally in Sheffield the assay was carried out using a dilution of porcine elastase of 0.1 mg/ml as 'stock'. In setting up this assay on microtitre plates for this study, we pursued the method exactly as originally described to us. This led to a deep colour change in all of the wells containing sample serum, indicating one of several problems with the assay technique. These, and the solution to the problem, are outlined below.

(a) Too much elastase enzyme - the most likely fault. If the enzyme was too concentrated it would fail to be inhibited by the relatively dilute samples, and act on the standard substrate load to produce the deep colour change obtained.

(b) No inhibition of elastase - unlikely option. For the initial setting up of the assay we were using standard samples of serum of known phenotype given to us by Sheffield. These had already been assayed by Sheffield, and the antitrypsin funtional assay result known.

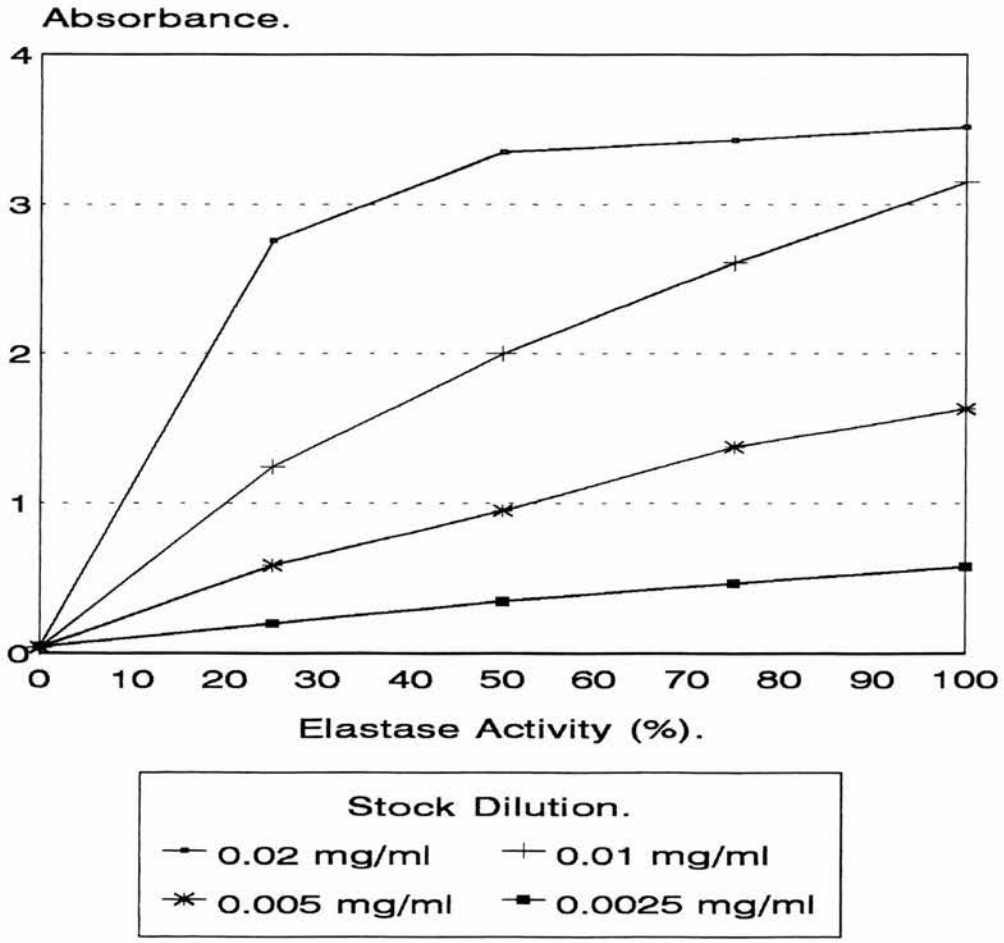
(c) Too much substrate - not likely either. Substrate availability is not a rate limiting step of the reaction.

In order to verify this, and to choose a suitable elastase concentration for our assay, we went on to produce calibration curves using known dilutions of elastase. 'Stock' elastase concentrations of 0.02 mg/ml, 0.01 mg/ml, 0.005 mg/ml, and 0.002 mg/ml were made up to replace the original 0.1

mg/ml concentration. Each of these stock solutions was then diluted in turn to 25%, 50% and 75% with TRIS buffer. When blank samples (TRIS only) and 100% samples (neat stock solution) were added to each series, this resulted in 0-100% dilutions of each of the four stock solutions, or 16 samples in all. The 16 samples were assayed as described above, and the results plotted as elastase activity vs. absorbance. The following graph was obtained (Figure 23). This resulted in the choice of 0.02 mg/ml as a working 'stock' elastase dilution, as any change in elastase activity early in the curve is accompanied by a relatively large change in absorbance.

Figure 23.

Dilutions of elastase used in calibration of the assay.



Having set up the assay, its sensitivity had also to be ensured. Standardisation of the assay in our laboratory was carried out using eight plasma samples from Sheffield of known antitrypsin phenotype and inhibitor assay. The eight samples used for standardisation were of mixed phenotypes for alpha-1-antitrypsin, there being two Z types, two MZ types one MS type, and two M types.

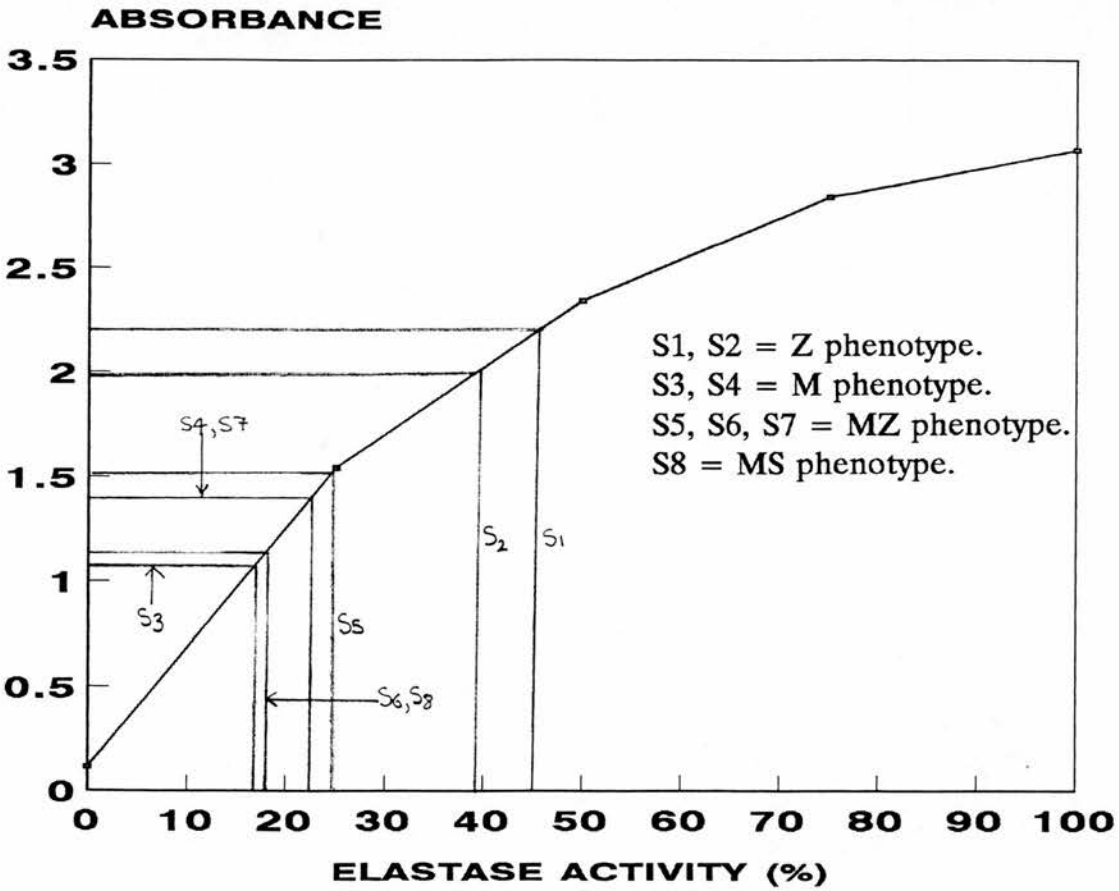
Using the method and reagent dilutions outlined and verified above, each of eight known samples were assayed. These included 2 x Z phenotype, 2 x M phenotype, 3 x MZ phenotype and 1 x MS phenotype. An adequate separation of phenotypes was sought. The results are shown below, with each phenotyped sample plotted and named (Figure 24). It can be seen that adequate separation of known phenotypes was obtained.

These results verified the standardisation of the assay, and its sensitivity in differentiating between samples of known and differing activity.

To ensure the standardisation of each assay run, three of the Sheffield Standards were assayed each time a batch of patient samples were assayed. This not only acted as a quality control, but provided results which could be used as an indirect check on reproducibility. In addition, a number of samples were used to assess the inter-assay plate co-efficient of variation. Results of reproducibility studies and patient sample assays are found in Chapter 8.

Figure 24.

Separation of antitrypsin phenotypes obtained using the assay described above.



Elastin Fragment Assay

This assay was carried out in the Laboratoire de Biochimie du Tissu Conjonctif, Faculté de Médecine, Université Paris Val de Marne, by kind permission of Dr. L. Robert. The method has not been published in any Journal, so is described here in detail.

Principle.

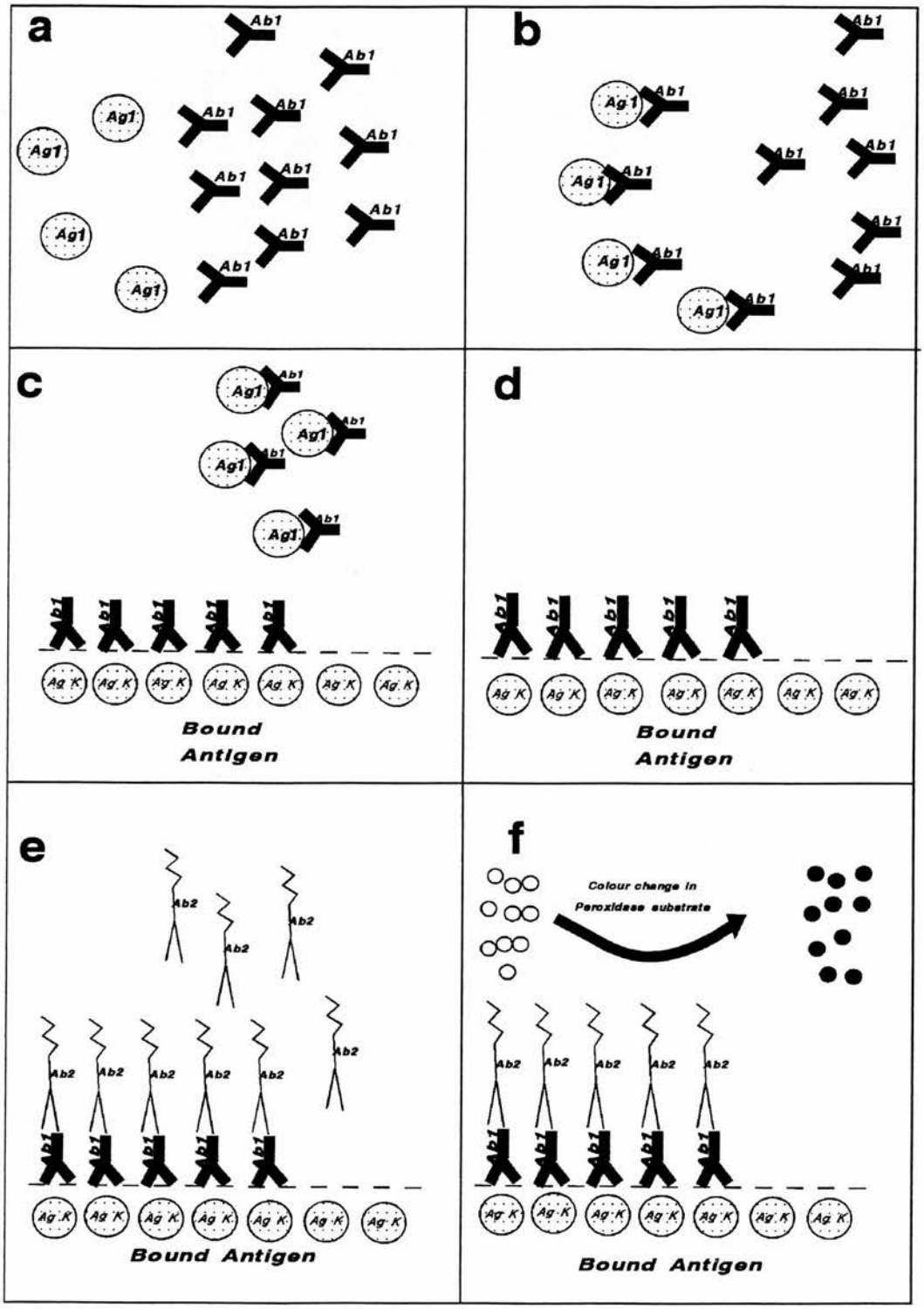
The principle of E.L.I.S.A. inhibition assay is illustrated in Figure 25, and references in brackets in the following paragraph refer to parts of that Figure.

The E.L.I.S.A. inhibition assay depends upon the interaction of specific antibodies to elastin fragments **(Ab 1)** and elastin fragments in patients plasma **(Ag 1)**. Fixed amounts of known dilutions of each are used, with an excess of antibody **(a)**. In this case, all of the elastin fragments in the patients plasma are bound by antibody, leaving a proportion of antibody 'free' **(b)**. This mixture is then exposed to further, synthetic elastin fragments **(Ag K)** bound on solid medium (the microtitre plate). Only antibody remaining free from the first reaction binds to the plate **(c)**. The amount of antibody binding to the plate is therefore inversely proportional to the amount of elastin fragments **(Ag 1)** in the patients plasma. The plate is then washed to remove all but antibody bound to synthetic elastin fragments attached to the plate **(d)**.

In order to assay the amount of antibody bound to the plate, a second antibody **(Ab 2)**, conjugated with peroxidase is used. This binds specifically to the first antibody **(e)**. A substrate for peroxidase is then used to produce a colour change measured by absorbance spectrophotometry **(f)**.

Figure 25.

The principle of E.L.I.S.A. inhibition assay



Patients.

This assay was carried out on a limited number of patients. The logistic difficulties of transporting frozen samples to Paris, and the expense of the assay meant that only 50 samples could be analysed. Those assayed included samples from 10 patients with inflammatory aneurysm, 20 with simple aneurysms, and 13 with aorto iliac occlusive disease. Seven samples spoiled in transit.

Procedure.

Preparation of Reagents

Reagents requiring preparation are **Ag K** - a preparation containing human elastin fragments, and **Ab 1** - an antibody against human elastin fragments.

Ag K is a heterogeneous mix of elastin peptides produced by the digestion of Human Aortic Elastin by potassium hydroxide. The elastin is incubated at 37°C with 1 molar KOH (20%)/Ethanol (80%) for 3.5 hours. The resulting preparation, also known as **Kappa Elastin** is made up in several dilutions in Phosphate Buffered Saline for coating microtitre plates.

Ab 1, also known as **first antibody**, is obtained by innoculating goats with Ag 1 (Kappa Elastin). It is an antibody against Kappa elastin, and therefore against human aortic elastin fragments.

Other reagents required are **Ab 2**, peroxidase substrate and a solid support medium.

Ab 2 or **second antibody** is available as Rabbit anti-goat-IgG conjugated with horseradish peroxidase (Miles Laboratories). The solid support medium used was plastic Micro-E.L.I.S.A. microtitre assay plates (Dynatech Laboratories). The peroxidase substrate was 1,2 phenylene diamine dihydrochloride.

Standardisation of Assay.

The sensitivity and reproducibility of the E.L.I.S.A. inhibition assay depends upon the concentration of Ag K used to coat the microtitre plate wells, and upon the concentration of Ab 1 used in the first part of the assay (see **a - c** in figure 25), both of which are determined empirically. It is for this reason that standardisation and calibration procedures are described before the procedure for patient sample assay.

From the laboratory's experience of performing hundreds of elastin fragment estimations, the two most frequently suitable concentrations of Ag K were found to be 0.4 μl/ml and 1.1 μl/ml.

Similarly, two suitable antibodies had been found for use as Ag 1. Both were polyclonal goat antibodies to Kappa elastin - SS3 and S13.

In order to determine the optimal combination of Ag K and Ab 1, the two dilutions of Ag K (0.4 μl/ml and 1.1 μl/ml) were each exposed to serial dilutions of each of the Ab 1 antibodies SS3 and S13. This required the

preparation of two microtitre plates - one coated with an Ag K concentration of 0.4 μg/ml, and one coated with a concentration of 1.1 μg/ml.

Each of these plates was treated in the same way, and exposed in one half to SS3 in serial dilutions, and in the other half to S13 in serial dilutions. In this way, four combinations of Ag K and Ab 1 were examined.

Further details of the preparation of the plates and other reagents are given below under the heading of 'Procedure 1'. Following preparation of the two plates described above, they were each exposed to Ab 2, and then peroxidase substrate, as described in 'Procedure 2' below.

Procedure 1.

1. Preparation of microtitre plates

100 μl Antigen at appropriate dilution added to each well. Plates incubated at 37°C for 2 hours, then maintained at 4°C until required. Immediately before use the plates are washed with Phosphate Buffered Saline containing Tween 20 (PBST) to stop non-specific protein binding.

2. Preparation of Serial Ab 1 Dilutions

100 μl of antibody added to 1 ml of PBST, to make dilution of 1/10. Serial dilutions then made in PBST to final dilution of 1/2560. This procedure carried out for each of the two enzyme systems SS3 and S13.

3. Preparation of Peroxidase Substrate/Buffer.

10 ml citrate/phosphate buffer 0.15M, pH 5.0

5 mg 1,2 phenylene diamine dihydrochloride

20 μl hydrogen peroxide.

4. Preparation of Ab 2.

65 μl rabbit anti-goat-IgG diluted in 10 mls PBST to final dilution of 1/1500.

Procedure 2

Plate washed with PBST and aspirated to dryness. 4 cycles.

According to worksheet, serial dilutions of antibodies added to plate. (100μl)

Incubated at 37^0 for 1 hour.

Plate washed with PBST and aspirated to dryness. 4 cycles.

Second antibody added to each well. (100 μl)

Incubated at 37^0 for 1 hour.

Plate washed with PBST and aspirated to dryness. 4 cycles.

Peroxidase substrate added to each plate. (100μl)

Plate incubated 37^0 for 15 minutes.

Reaction stopped by addition of 50 μl of 2M hydrochloric acid to each well.

The light absorbance of each well read at 490 nm using Dynatech 5000 spectrographic microtitre plate reader.

The optimal coating antigen concentration and first antibody titre combination was chosen by selecting a combination which gave a final optical density at 490 nm of 1.1 - 1.3. A combination of 0.4 μg/ml of coating antigen and 1/100 SS3 first antibody was found by this method to be most suitable.

Henceforth, **Ag K** shall refer to a solution of **0.4 μg/ml of Kappa elastin.**

Ab 1 will refer to a **1/100 dilution of SS3 antibody** (goat anti-kappa-elastin)

Assay of Patient Samples and standards.

Five microtitre plates were coated using the technique descibed above. An Ag K concentration of 0.4 μg/ml was used, having been found to be optimal.

Five separate, corresponding plates were prepared. These were not coated, but served to mix patients plasma with Ab 1 before addition to the coated plate. The plate assignment was chosen to provide 12 assays of plasma each in triplicate. Rows were reserved for serial dilutions of antigen to produce a standard curve for each plate.

Serial dilutions of Kappa elastin were prepared in order to produce a standard curve. 132μl of Kappa elastin (2 mg/ml) was added to 1068 ml of bovine serum albumin (60 mg/ml in PBS), to make a total volume of 1.2 ml. Serial 1/3 dilutions of this were made X 11, to give 12 known concentrations of Kappa elastin.

60 μl of samples of plasma, or 60 μl of standard dilutions were added to each well according to the worksheet.

60 μl of first antibody at 1/50 dilution was then added to each well, giving a final first antibody concentration of 1/100.

The plates were covered and incubated at 37° for 1 hour, following which transfer of aliquots of each antibody/antigen mixture were made from the mixing plates to the **coated** microtitre plates.

Aliquots of 100 μl from **each well** of the mixing plates were transferred to corresponding wells in the **coated plates**. The mixing plates were discarded. The coated plates were covered and incubated at 37° for 1 hour, following which they were washed and aspirated to dryness for 4 cycles.

100 μl of 1/1500 dilution of second antibody was added to each well, and the plates incubated as before for 1 hour. They were once again washed and aspirated to dryness for 4 cycles.

100 μl of peroxidase substrate was added to each well, and the plates incubated at 37°C for 15 minutes, after which the reaction was stopped by the addition of 50 μl of 2M hydrochloric acid to each well.

The optical density of each well was read and recorded by the Dynatech microtitre plate spectrophotometer. Resident software in the Dynatech machine drew the standard concentration curve represented by standard antigen samples, and calculated the equation of the line. Using this, it also calculated the concentration of antigen (elastin fragments) in the 'unknown' samples. The mean elastin fragment concentrations for each sample were calculated from the triplicate readings.

Results were stored on computer for later analysis, and can be found in Chapter 8. Because of time and financial constraints in the performance of this assay, no reproducibility data was collected.

Alpha-1-Antitrypsin Phenotyping

Principle.

Isoelectric focussing was used to determine the antitrypsin phenotype present in the serum of each study patient. This method is well established, and is the standard technique used for antitrypsin phenotyping.

Enzymes, as proteins, possess a large number of chemical groups which are capable of existing in different ionic forms. Some of these groups are situated on the outside of the protein molecule, and respond to differences in pH by altering their charge state.

Isoelectric focussing is a form of electrophoresis in which an electric current is applied to a protein mixture dissolved in a suitable medium in a pH gradient. The proteins separate according to their isoelectric points.

The medium used for electrophoresis of antitrypsin phenotypes is agarose gel. The gel is placed between two solutions of differing pH values; an anolyte (at the anode end of the gel) and a catholyte (at the cathode end). This provides the pH gradient. Samples of patient and standard serum are placed in the gel, and an electric current passed through the gel. When focussing has taken place, the agarose gel is removed, and stained to display the positions of the focussed samples. The principle is illustrated in Figure 26 below.

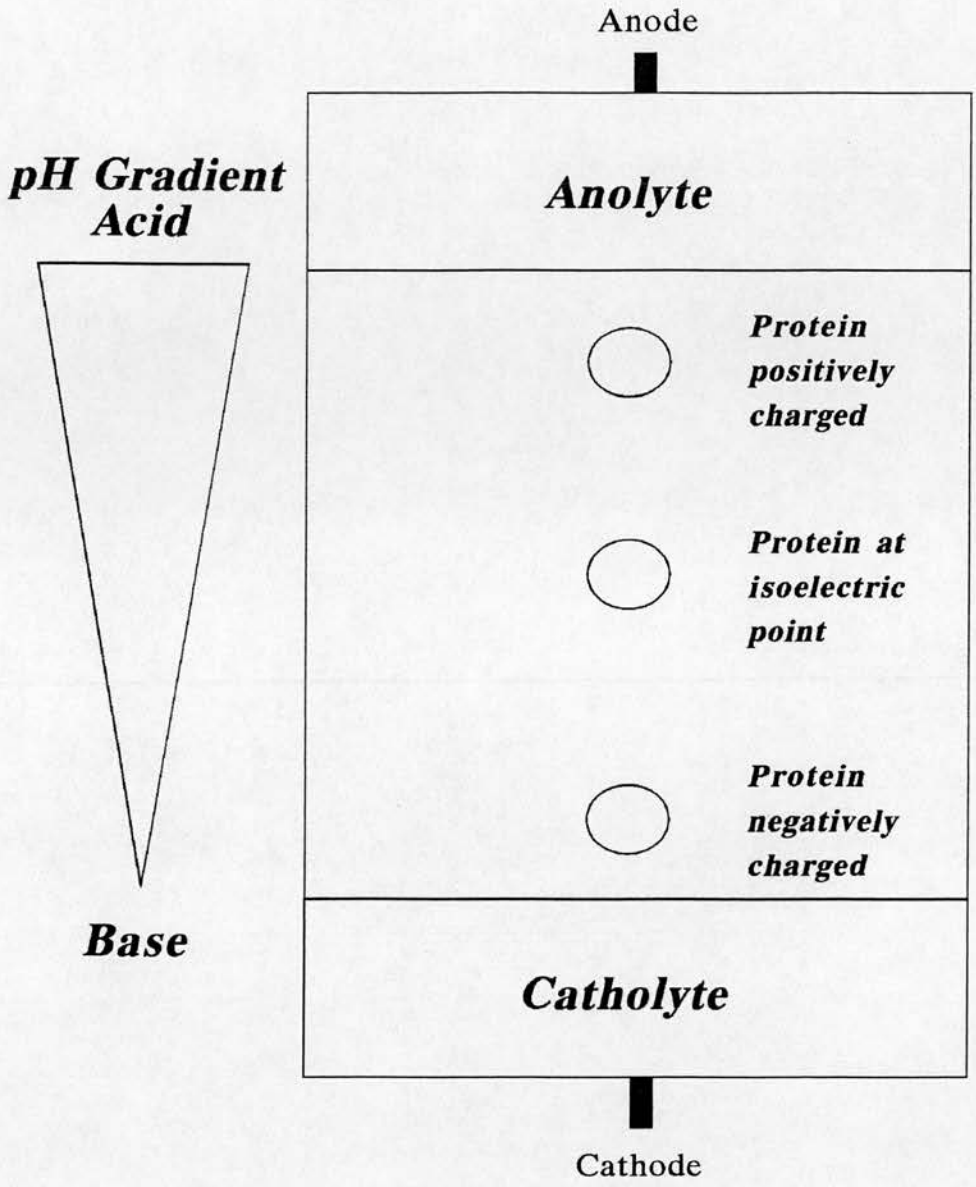


Figure 26.

The principle of isoelectric focussing applied to alpha-1-antitrypsin (a-1-at). A pH gradient is created in a gel sheet ranging from acidic to basic. Samples of patients serum are placed at the basic end of the gel, giving the contained a-1-at molecules a net negative charge. When a voltage is applied across the gel (anode at the acidic end, cathode at the basic end) the a-1-at moves toward the anode. In moving through the pH gradient, the net charge on the a-1-at changes, becoming more positive until there is no net charge; it therefore stops migrating through the gel. The pH (and therefore the point on the gel) at which this occurs is the *isoelectric point*. As this differs for each phenotype, each stops at a different point on the gel.

Patients.

Antitrypsin phenotyping was performed on stored samples of serum from 15 patients with inflammatory aneurysms, 36 with simple aneurysms, and 33 with aorto-iliac occlusive disease.

Procedure.

Preparation of reagents.

Gel preparation.

0.259g IEF agarose (Pharmacia 17-0468-01)

2.03g sucrose

0.075g Aminoethyl sulphonic acid (Sigma A9758)

15 ml distilled water

Place in boiling water bath until completely dissolved, then cool to 65°C before adding 1 ml of Pharmalyte pH 4.5-6.5 (Pharmacia 17-0452-01)

Pour into pre-heated casting plates using 0.75mm former, and cool in refrigerator at 4°C for at least 1 hour.

Sample preparation.

Patient samples stored in -80°C freezer until required, then .

Immunofixation - use serum diluted 1:3 with distilled water.

Gel staining - use neat serum.

Assay of Patient samples and standards.

Anolyte - 0.05 molar sulphuric acid.

Catholyte - 1 molar sodium hydroxide.

Allow the gel to come to room temperature, before placing in the isoelectric focussing (I.E.F.) tank. Apply samples and standards 1.5 cm from cathodic edge of gel, and allow to soak into gel.

Soak wick in catholyte, and place on cathodic edge of gel.

Soak wick in anolyte, and place on anodic edge.

Close tank and apply current (8mA) between anode & cathode wicks.

Focussing takes approx. 2 hours.

Gel Staining

Fixative - 17.3g sulphosalicylic acid
- 57.5g trichloroacetic acid
- 500 ml distilled water

Destain - 1125 ml methanol
- 250 ml glacial acetic acid
- 1125 ml distilled water

Place focussed gel in fixative for 15 minutes, then press dry, and wash twice with destain for 5 minutes each time. Press dry and stain with Page Blue stain for 10 minutes. Destain and dry.

Immunofixation.

Immunofixation was used when simple gel staining was unable to provide adequate definition of phenotypes. This relies on a combination of a specific antigen antibody reaction between the antitrypsin sample on the agarose gel and subsequent staining of the antigen-antibody complex rather than simple nonspecific staining of protein. The antibody used was Atlantic Antibodies alpha-1-antitrypsin antiserum.

Dilute 0.5 ml of antiserum with 0.5ml of 8% polyethylene glycol-buffered saline, mix and soak into strip of cellulose acetate wide enough to cover sample strips to be immunofixed.

Place acetate strip on gel, and leave for 20 minutes.

Remove acetate, and press gel dry for 5 minutes.

Place gel in stirred saline bath of 2 hours to remove all protein not bound to antiserum.

Press gel dry, and stain in Page Blue stain for 5 minutes.

Destain and dry gel.

Standardisation of Assay.

Standard samples of MM, MZ, MS, and ZZ phenotype sera kept in -80°C freezer until use. At least 1 standard sample of each phenotype included in each focussing run.

Results of this assay were stored for later analysis, and can be found in Chapter 8.

RADIOLOGICAL INVESTIGATIONS.

Ultrasound examinations were carried out using either an Advanced Technology Laboratories Mk. 600 scanner with a 3.75 MHz. mechanical sector scanning head or a Toshiba SSA-250A scanner with a 3.75 MHz. curvilinear array head. Each scan was performed by a consultant or senior registrar in radiology with a special interest in vascular imaging.

C.T. Examinations were carried out using a dynamic contrast enhanced scan technique on a Seimens Somatom DRH C.T. scanner. The slice width was 8 m.m., with a 20 m.m. slice gap. Patients were scanned from xiphisternum to pubis, according to the likely extent of the aneurysm.

M.R.I. images were acquired using a Picker Vista MR2055 scanner operating at 0.5 Tesla with a helium/nitrogen supercooled magnet.

The images were obtained using saggital and coronal spin echo acquisition (Time to echo [TE] 26, Repetition time [TR] 500-600 msec.) Transverse images were ungated multi-echo and S.T.I.R. sequences. The multiecho sequences had first-echo images at TE 20, TR 1800, the second image at TE 80, TR 1800. The S.T.I.R. sequences were acquired with a TE of 30, Time to inversion (TI) 100, and TR of 1500. On the majority of the images, the field of view was between 40 and 45 cm, slice thickness was 10 m.m.

Parameters used for patient examination were used in in-vitro specimen examinations.

CHAPTER 7.

RETROSPECTIVE SERIES RESULTS

INTRODUCTION

The following Chapter contains the results of each investigation in the retrospective study. The following are dealt with:

Clinical details

Symptoms of disease

Pre-operative investigations

Operative findings

Operative technique

Post-operative course

RETROSPECTIVE PATIENTS

Clinical Details.

A total of 47 patients with inflammatory aneurysms and 162 with non-inflammatory aneurysms were included in the retrospective study.

Age and Sex of retrospective series.

Disease Type	Mean Age	Sex
Inflamm. Aneurysm (n=47)	70 (Female) 66 (Male) Range 46-85	5 Female
Simple Aneurysm (n=162)	69 (Female) 70 (Male) Range 46-85	18 Female

Concomitant disease was also surveyed in the retrospective populations, and the details of these are given in the tables below. Diabetes was regarded as present if the patient was under treatment by diet, oral hypoglycaemic drugs or injected insulin. A history of claudication, myocardial infarction, angina pectoris or stroke (or T.I.A.) was taken as evidence of occlusive vascular disease.

Concomitant disease in retrospective patients.

Disease type	Diabetes	Ex/Current Smokers	Occlusive vascular disease
Inflamm. Aneurysm	0	47 (100%)	18 (38%)
Simple Aneurysm	4 (2%)	140 (86.5%)	63 (38%)

These differences did not reach statistical significance.

Systolic and diastolic blood pressures did not differ between the groups, nor did the incidence of known or apparent hypertension. This was taken as either a documented history of hypertension with or without treatment, or a persistently raised diastolic blood pressure of > 100 mm.Hg. In the following tables, means are given, with standard deviation (SD)

Blood Pressure in retrospective patients.

Disease Type	Systolic B.P.(mmHg)	Diastolic B.P.(mmHg)	Known or Apparent Hypertension
Inflamm. Aneurysm	148 SD 21.6 Range 100-200	90 SD 13.3 Range 70-120	25 (53%)
Simple Aneurysm	150 SD 26.0 Range 110-260	88 SD 16.3 Range 50-150	91 (56%)

Pre-operative investigations 1. Symptoms of disease.

With regard to symptoms of disease, 43% of patients with inflammatory aneurysms were discovered to have aneurysmal disease incidentally during investigation for other pathology, and 56% presented because of symptoms caused by their aneurysm. Pain in the abdomen or back was a significant feature of the history in 82% of cases.

Pain was only a feature of the history in 56% of cases in the group with simple aneurysms, significantly fewer than in the inflammatory group, ($p<0.001$, X^2)

The presence of abdominal or back pain had a sensitivity of 82% for the detection of inflammatory aneurysm, and a specificity of 44%. The Positive Predictive Value was 0.31, and the Negative Predictive Value 0.88.

There was no difference in the stated incidence of weight loss between the two groups, (22% inflammatory vs. 36% non-inflammatory)

Eight patients displayed symptoms of systemic illness, with malaise, anorexia and weight loss. All of these patients had a plasma viscosity above 1.75 mPa. In six cases, other organs were involved in fibrosis in addition to the duodenum (ureters in 3 cases, left renal vein in 2, and inferior mesenteric vein in 1.

In the three cases with ureteric involvement, one had bilateral hydronephrosis, and two had unilateral hydronephrosis, and all required operative ureterolysis.

Pre-operative investigations 2. Haematology and biochemistry

Haematological parameters were also measured, and are shown below as means and standard deviation (SD). The plasma viscosity value was significantly greater in the inflammatory group, using the Mann-Whitney u-test.

Haematological parameters in retrospective patients.

Disease Type	Hb (x10 /l)	WCC (x10 /l)	Platelets (x10 /l)	Plasma Viscosity
Inflamm. Aneurysm	13.1 SD 1.68	8.9 SD 2.2	319 SD 98	1.84 SD 0.19
Simple Aneurysm	13.0 SD 1.7	7.7 SD 2.2	248 SD 68.9	1.70 SD 0.13

As a practical measure, the numbers in each group were tabulated for plasma viscosity above 1.72 mPa. When subject to Chi-square testing, the inflammatory group showed a significantly greater number of patients with raised viscosity ($0.001 > p > 0.01$).

Raised plasma viscosity therefore had a sensitivity of 75%, and specificity of 56% in the detection of inflammatory aneurysm. The Positive Predictive Value was 0.42, and the Negative Predictive Value 0.84.

Pre-operative biochemical disturbance compatible with poor renal function, as defined by a plasma creatinine over 120 μmol/l and/or a creatinine clearance under 40ml min m^2 with appropriate electrolyte disturbance, was present in 9 patients in the inflammatory group. This failed to predict involvement of the ureters in the inflammatory process.

Ten patients underwent ureterolysis as part of their operative procedure. Nine of these patients had no preoperative biochemical evidence of renal failure or electrolyte disturbance.

Pre-operative investigations 3. Radiology

Rapid sequence contrast enhanced abdominal C.T. was carried out in 25 of the 47 patients with inflammatory aneurysms. This failed to make the diagnosis of inflammatory change in 12 cases. Abdominal ultrasound failed in all cases, as did plain abdominal radiography.

Operative Findings

At operation, other structures were involved in the inflammatory mass in every case. In all cases of abdominal inflammatory aneurysm the duodenum was adherent to the aneurysm wall. Ten patients had involved ureters, 3 dense peri-aortic adhesions, 2 stenosed IVC, and 5 involved left renal vein. In 1 case, the inflammatory aneurysm was solely thoracic.

Operative Technique

In 42 cases of inflammatory aneurysm, operative replacement of the aneurysmal aorta was carried out transperitoneally using the inlay technique. The single thoracic aneurysm was resected through an eighth rib thoracotomy.

In the remaining 4 cases, extension of the inflammatory process up to or above the diaphragm required extension of the abdominal incision to a full thoraco-abdominal approach for supradiaphragmatic aortic clamping. In all 4 cases, aneurysmal dilation was confined to the infrarenal aorta but involved the area immediately adjacent to the renal vessels making exposure from the front impossible.

Cases 1+2:

Encasement of the left renal vein in the inflammatory process precluded safe infradiaphragmatic dissection and clamping of the aorta. Following supradiaphragmatic control of the aorta in the thorax, using an eighth rib thoraco-abdominal incision, the aortic prosthesis was anastomosed to the infrarenal aorta.

Cases 3+4:

The inflammatory change was found to extend to include the origin of the superior mesenteric artery. Aortic clamping was carried out using the same site and technique as for cases 1 & 2, and the prosthesis anastomosed to the thoracic aorta.

Operations involving abdominal inflammatory aneurysms took significantly longer than for simple abdominal aneurysms taking an average of 3.4

hours to complete, compared to 2.9 hours for simple aneurysms ($p<0.005$, Mann-Whitney). Significantly more blood (2.9 litres vs. 1.6 litres $p<0.001$, M-W) and colloid (1.6 litres vs. 1.1 litres $p<0.05$, M-W) was infused per-operatively in the case of inflammatory abdominal aneurysms.

36% of patients with inflammatory aneurysms underwent tube grafting, while 63% had bifurcated grafts. This constituted a significantly higher incidence of bifurcation grafting than in the non-inflammatory group, (66% tube grafts, 34% bifurcated. $p<0.001$, X^2 test).

Post-operative course

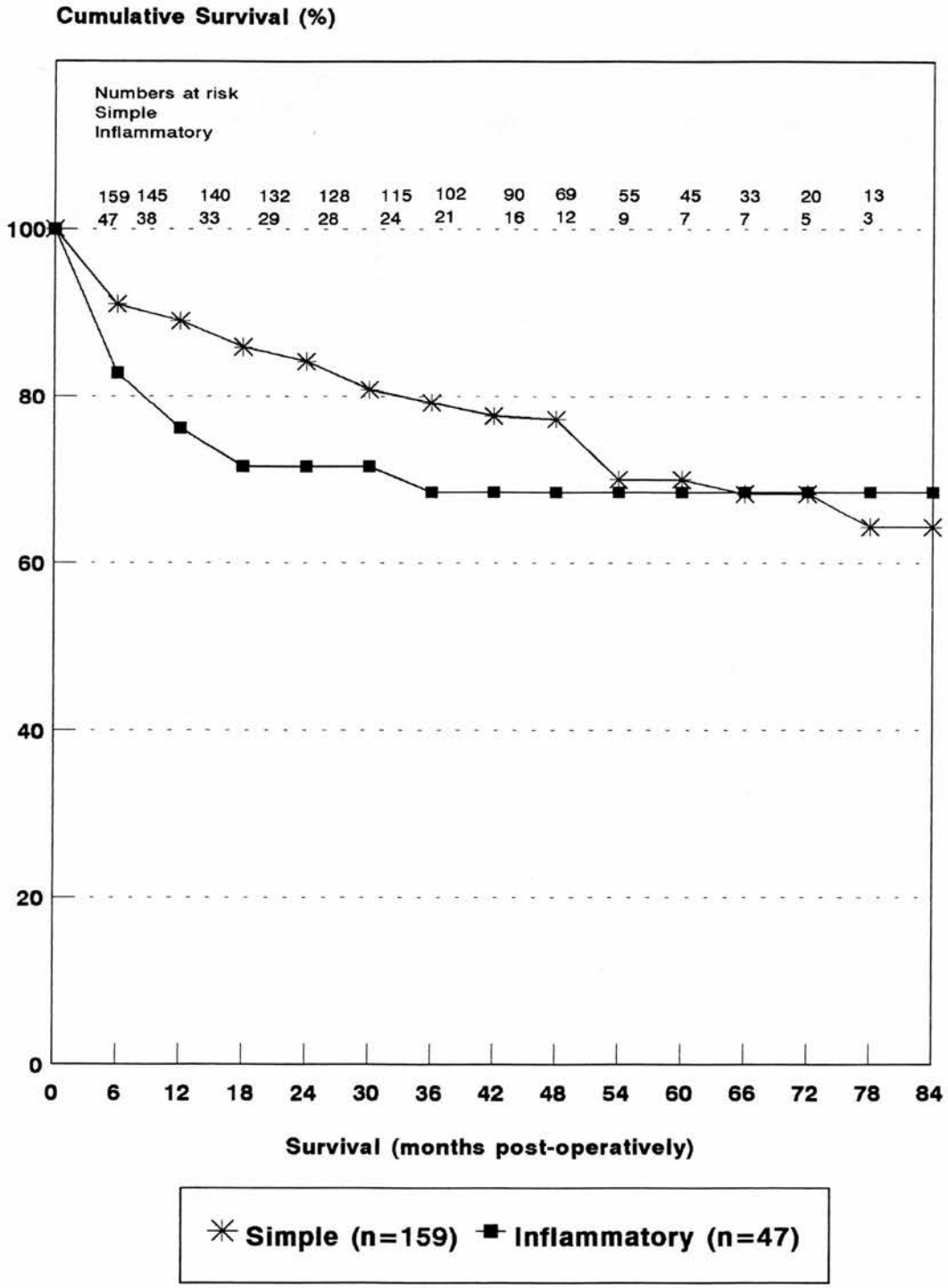
The 30 day mortality for inflammatory aneurysms was 13% (6 patients), with another two deaths within 6 months of operation. The major cause of death within 30 days was myocardial infarction (4 patients). Pulmonary embolus (1), and acute renal failure (1) accounted for the other two deaths. One of the further deaths occurring within 6 months was due to myocardial infarction; the cause of the other is unknown.

The hospital mortality of the non-inflammatory group was 4.3%. The cause of death was myocardial infarction in 5 cases, with acute renal failure and intractable left ventricular failure accounting for one each.

Life table analysis of patients with simple and inflammatory aneurysms can be seen in Figure 25 (overleaf). No follow-up data were available for 3 patients.

Figure 27.

Life table analysis of post-operative survival of patients in the retrospective series.



SUMMARY.

The results of this prospective study have shown that inflammatory change continues to complicate a significant proportion of all aortic aneurysms.

Inflammatory aneurysms are indistinguishable from their simple counterparts on grounds of age, risk factors or symptoms. Routine biochemical investigations are of no benefit, and do not predict entrapment of the ureters, one of the major complications of inflammatory aneurysms. The only haematological investigation differing between simple and inflammatory disease is the plasma viscosity, but even this is not sufficiently sensitive to act as a diagnostic test.

Radiological investigation was equally unsuccessful. Ultrasound failed in all cases to reveal the presence of inflammatory change, while contrast-enhanced computerised tomography made the diagnosis in just over 50% of subsequently confirmed cases.

Findings at operation for inflammatory aneurysms confirmed the presence of a high incidence of involvement of other organs such as the ureters and duodenum. This was associated with longer operation times, increased blood and colloid requirement, and increased use of bifurcated grafts. The necessity for major intra-operative changes in technique to deal with unsuspected inflammatory change has been illustrated.

Although the long term post-operative survival of patients with inflammatory and simple aneurysms is similar, operative mortality is increased in cases of inflammatory aneurysm.

CHAPTER 8.

PROSPECTIVE STUDY RESULTS

INTRODUCTION.

The following Chapter contains the results of the investigations carried out on the prospectively studied series of patients.

As with the preceding Chapter, the first part of this Chapter is concerned with the following:

Clinical details

Symptoms of disease

Pre-operative investigations

Operative findings

Operative technique

Post-operative outcome

The second part of the Chapter is concerned with the results of the original biochemical investigations detailed in Chapter 5. It includes both results of reproducibility studies and results of patient samples.

The chapter goes on to describe the results of the radiological investigations.

Clinical Details

The prospective study group consisted of 15 patients with inflammatory aneurysms, 83 patients with simple, non-inflammatory aneurysms, and 37 patients with severe aorto-iliac occlusive disease. Their age and sex distribution is shown below.

One patient in the 'inflammatory' group died pre-operatively of ruptured aneurysm, having undergone all pre-operative investigations. Post mortem confirmed the inflammatory nature of the aneurysm. For the sake of clarity, other post-mortem findings are included in the 'operative findings' section.

Age and Sex Distribution - Prospective Patients.

Disease Type	Mean Age (yrs)	Median Age (yrs)	Sex
Inflamm Aneurysm	67.36 $^{\text{Range } 53\text{-}80}_{\text{SD } 7.8}$	67.14	2 Females
Simple Aneurysm	72.22 $^{\text{Range } 55\text{-}88}_{\text{SD } 7.3}$	73.47	11 Females
Occlusive Disease	64.62 $^{\text{Range } 55\text{-}78}_{\text{SD } 8.9}$	65.24	5 Females

Other patient characteristics are summarised in the succeeding pages.

Risk Factors in Patient Groups.

Disease Type	Diabetes	Smokers	Known Hyper-tension	Systolic B.P.	Diastolic B.P.
Inflamm. Aneurysms	0	13	5	163 Range 130-220 SD 32.9	93 Range 80-120 SD 13.4
Simple Aneurysms	5	68	28	151 Range 110-220 SD 23.7	89 Range 70-120 SD 12.35
Occlusive Disease	4	37	7	146 Range 100-190 SD 21.9	83 Range 40-110 SD 14.36

Diabetics include those controlled by diet alone, oral hypoglycaemics, and insulin. Smokers are those patients who are either current smokers or have stopped smoking in the last 5 years. In the case of hypertensives, patients are regarded as such if they are under treatment for hypertension, or were found to be so consistently pre-operatively, but were untreated before admission. Mean blood pressures are calculated from readings for each patient taken in the ward pre-operatively when measurement had been consistent on three occasions.

Symptoms of Disease

While only 26% of inflammatory aneurysm patients were discovered to have aneurysms incidentally during investigation for other illnesses, this group accounted for 67% of patients with simple aneurysms. All other patients in each group presented with symptoms directly attributable to their aneurysm.

Pain was a feature in 80% of inflammatory aneurysms, but was present in only 45% of simple aneurysm patients, while weight loss occurred more frequently in inflammatory aneurysm patients (53%) than in patients with simple aneurysms (29%).

Pre-operative investigations

Haematology Results.

Disease Type	Hb. (g/dl)	White Cell Count. (x 10^9/l)	Platelet Count. (x 10^9/l)	Plasma Viscos. (mPa)
Inflamm. Aneurysm	12.66 Range 7.5-15.4 SD 2.27	8.19 Range 5.7-10.9 SD 1.93	295 Range 185-482 SD 104.7	1.79 Range 1.52-2.01 SD 0.2
Simple Aneurysm	13.24 Range 8.5-16.3 SD 1.65	8.06 Range 4.4-20.3 SD 2.93	229 Range 41-485 SD 71.23	1.65 Range 1.37-2.04 SD 0.12
Occlusive Disease	13.30 Range 8.9-16.7 SD 1.7	8.77 Range 5.0-18.0 SD 3.01	274 Range 141-443 SD 69.64	1.64 Range 1.41-1.97 SD 0.12

Plasma Viscosity - Contingency Tables.

Plasma Viscosity	Inflamm. Aneurysm	Simple Aneurysm	Occlusive Disease
> 1.72 mPa	9	21	8
< 1.72 mPa	6	62	29

There was no statistical difference between the groups in pre-operative hæmoglobin or total white cell count. Platelet counts for the simple aneurysm group were significantly lower than the other groups ($p < 0.02$ in each case,

Mann-Whitney). Mean plasma viscosity was significantly raised in the group of patients with inflammatory aneurysms compared to the other two groups ($P < 0.03$ Mann-Whitney in both cases). Chi-square testing for a viscosity over 1.72 mPa showed a significantly increased incidence in the inflammatory group over the other two groups with a p value of $0.01 < p < 0.05$.

Using the same criteria as in chapter 7 to define renal functional compromise, that is either a serum creatinine above 120μmol/l or a creatinine clearance below 40 ml/min with appropriate electrolyte disturbance, 6 of the 15 patients with inflammatory aneurysms displayed some compromise. This compares with 11 of the 83 patients with simple aneurysms. Of the six patients in the inflammatory group, only 2 had ureteric involvement in fibrosis.

Operative Findings.

In the 15 cases of inflammatory aneurysm, 6 patients had involvement of the ureters either unilaterally or bilaterally, while the inferior vena cava was involved in three cases, and the left renal vein in three.

Operative Technique.

The transperitoneal abdominal approach was used in all but three cases of inflammatory aneurysm. In two cases, the aneurysm was solely thoracic, and in the other, the aneurysm was suprarenal but below the diaphragm, and a thoraco-abdominal approach was made.

In the group of simple aneurysms, 5 aneurysms were approached through a thoraco-abdominal incision. Two were solely thoracic, and two

extended proximally to the diaphragm. The fifth extended from the lower thoracic aorta to the mid-abdominal aorta.

The remaining aneurysms were confined to the abdomen, and were approached transperitoneally.

Of those undergoing resection of an abdominal aneurysm in each group, it was possible to insert a tube graft in 54% of patients with simple aneurysms, but only 23% of patients with inflammatory aneurysms.

Six patients with inflammatory aneurysms had either unilateral or bilateral involvement of the ureters in fibrosis. Only two of these patients had hydronephrosis, but all underwent ureterolysis.

The results for intra-operative blood loss are as follows, excluding patients in whom a thoraco-abdominal approach was used:

Intra-operative Blood Loss & Transfusion - Transperitoneal Approach.

Disease Type	Blood Loss (ml)	Blood Transfused (ml)	Colloid Infusion (ml)	Crystalloid Infusion (ml)	Operative Time (hrs)
Simple Aneurysm	1900 Range 500-4400 SD 1022.2	1984 Range 500-6000 SD 1143.5	1591 Range 500-4000 SD 741.5	1750 Range 1000-3000 SD 717.6	3.85 Range 1.5-6.5 SD 1.5
Inflamm. Aneurysm	4160 Range 1110-13000 SD 3632.6	3454 Range 1500-10500 SD 2494.5	2172 Range 500-4000 SD 1314.6	1600 Range 1000-3000 SD 699.2	4.18 Range 3-7.5 SD 1.26

Values for blood loss and blood transfused are significantly greater for operations involving inflammatory aneurysms than simple aneurysms ($p < 0.02$, Mann-Whitney).

Differences in other values do not reach statistical significance.

Very extreme values for blood loss and blood transfusion are found in one of the cases of inflammatory aneurysms. If this case is excluded, figures for blood loss and blood transfusion remain significantly greater in the inflammatory group. Mean blood loss falling to 3177 ml, and blood transfusion to 2750 ml.

Post-operative Outcome

1. - Simple aneurysms

The post-operative mortality will be dealt with separately for patients undergoing transabdominal resection of simple aneurysms, and for thoraco-abdominal approaches. The 30 day mortality for the 78 patients undergoing transabdominal resection of simple aneurysms was 7.6% (six patients of 78). Causes of death were:

Myocardial infarction (3)

Acute renal failure (2)

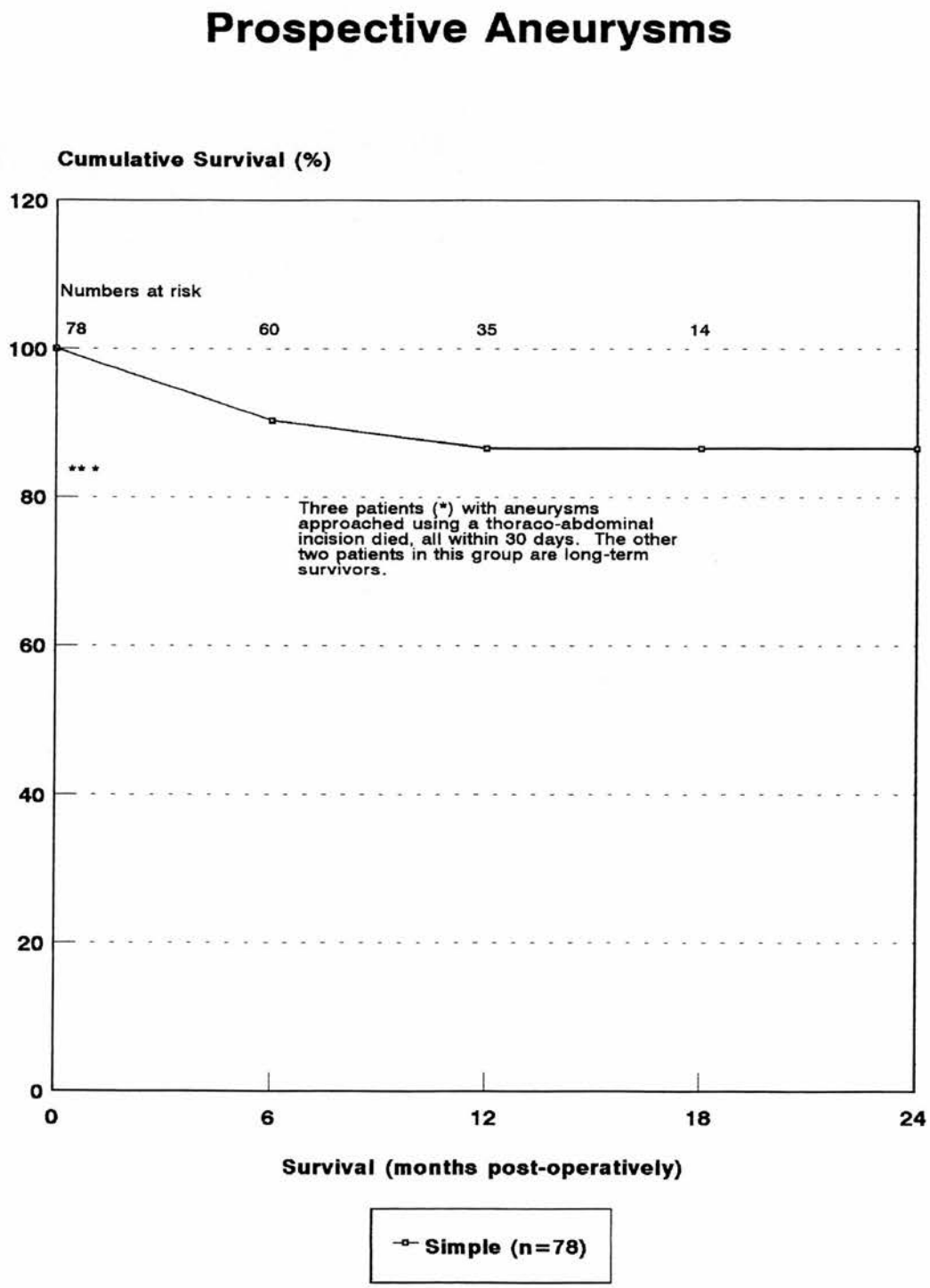
Septicæmia (1)

There was only one other death within 6 months, but the cause in this case is unknown. The overall six month mortality for this group was therefore 8.9%.

Life table survival analysis for patients with simple aneurysms resected transabdominally is shown below (Fig 28). The survival of the 5 patients with aneurysms resected using a thoracoabdominal approach are marked separately. 3 of the 5 died within 30 days of operation: 2 of myocardial infarction, 1 of acute renal failure. 2 are still alive.

Figure 28.

Life table analysis for prospectively studied simple aneurysms. Those resected using a thoracoabdominal approach are shown separately.



2. - Inflammatory aneurysms

One patient died pre-operatively, and excluding thoraco-abdominal approaches, this leaves 11 patients to consider, with transabdominally resected inflammatory aneurysms. None died within 30 days, and only 2 within 6 months. One death was due to renal failure, the other cause of death was unknown. Of three patients undergoing thoracoabdominal resections of inflammatory aneurysms, two died: One at 2 days post-operatively of a stroke, the other at 121 days of myocardial infarction.

Neutrophil Elastase Assay.

In all cases, assays were carried out in duplicate. Manufacturers Batch numbers for each kit varied, and the elastase concentration of "standard" and "control" sera was not constant between all kits. There was therefore potential for variation in a number of factors, including also assay tube coating and substrate concentration.

In order to assess the reproducibility of the assay between kits, 22 samples selected as detailed in 'Methods' (above) were assayed in two kits each. Each kit contained at least two 'repeat' samples.

The co-efficient of variation between assays was calculated as detailed in: *Penberthy L A, A users Guide to Clinical Chemistry. Clin. Biochem. Revs. 1986;7:39-47.*

1. Inter-kit reproducibility.

Sample Number	Elastase 1(μg/l)	Elastase 2(μg/l)	Difference Squared
1	50.73	50.12	0.372
2	44.12	37.02	50.41
3	53.7	55.38	2.82
4	79.72	67.87	140.42
5	154.89	142.87	144.48
6	81.45	81.2	0.0625
7	248.45	231.77	278.22
8	85.33	68.98	267.32
9	70.03	63.4	43.95
10	61.70	65.46	14.13
11	68.59	67.32	1.61
12	92.7	90.25	6.00
13	104.9	93.62	127.23
14	44.92	45.23	0.096
15	35.75	39.03	10.75
16	70.23	77.73	56.25
17	45.18	47.39	4.89
18	53.73	50.12	13.03
19	52.16	58.24	36.96
20	64.3	67.58	10.75
21	101.02	103.05	4.12
22	53.58	55.29	2.92

For this data,

Value mean ($\hat{x}_v$) - 76.72
Σ_{s^2} - 1216.79
n=44

Co-efficient of variation calculated as

$$CV = \frac{\sqrt{\frac{\Sigma_{s^2}}{n}}}{\hat{x}_v}$$

$$CV = \frac{\sqrt{\frac{1216.79}{44}}}{76.72}$$

CV = 6.8%

2. Control Plasma Values (μg/l)

Kit No.	Known value	Calculated Value	% Difference
*1	125	169	35.2
*2	60	72.24	20.4
*3	60	75.77	26.2
4	135	145.99	8.1
5	135	128.24	5.0
6	60	65.61	9.3
7	60	66.62	11.0
8	60	65.75	9.5
9	60	64.37	7.2
10	60	55.99	6.6
11	60	61.91	3.1
*12	60	45.86	23.5
13	55	52.04	5.3
14	60	56.53	5.7
15	55	59.79	8.7
16	55	61.6	12.0

Kits marked with * were deemed inaccurate, and results not included in study

Summarised data for all patient groups are given here. The statistical significance values given use the Mann-Whitney u-test in each case.

Because of the presence of extreme values in the elastase results for inflammatory aneurysms, means and significances have been calculated with and without the two highest values of 124 and 248 μg/l. Calculation without extremes reduces the co-efficient of variation and standard error of values for inflammatory aneurysms to values considerably closer to the other two groups.

Elastase Assays - Including Extreme Results.

Disease Type	Mean (μg/l)	Standard Deviation	Standard Error	Co-eff of var.	Number
Inflamm. Aneurysms	88.15	49.35	12.74	55.99	15
Simple Aneurysms	59.69	25.29	3.11	42.36	66
Occlusive Disease	61.04	32.52	5.75	53.28	32

Comparison of groups using Mann-Whitney U-test gives the following results:

Inflammatory vs. Simple aneurysms -
$U = 248$, $z = -3.0031$, $p = 0.003$

Inflammatory aneurysms vs. Occlusive disease -
$U = 117$, $z = -2.8072$, $p = 0.005$

Simple aneurysms vs. Occlusive disease -
$U = 1011$, $z = -0.3409$, $p = 0.732$

Calculation of the same parameters without the two extreme values in the inflammatory group is as follows:

Elastase Assays - Excluding Extreme Results.

Disease Type	Mean (μg/l)	Standard Deviation	Standard Error	Co-eff of var	Number
Inflamm. Aneurysms	73.00	18.44	5.11	25.26	13
Simple Aneurysms	59.69	25.29	3.11	42.36	66
Occlusive disease	61.04	32.52	5.75	53.28	32

Comparison of the groups, again using the Mann-Whitney U-test Gives -

Inflammatory vs. simple aneurysms:
$U = 247$, $z = -2.4064$, $p = 0.016$

Inflammatory aneurysms vs. occlusive disease:
$U = 116$, $z = -2.3040$, $p = 0.021$

Elastin Fragment Assay

Elastin Fragments were assayed as described above. Limited numbers from each patient group were assayed because of restrictions in the time and facilities available to perform the assay. Elastin fragment assay was performed in the University of Paris, Créteil, Paris. It was made possible by the kind permission of Dr. L. Robert, Director of Research, with whom Dr. Marie-Paule Jacob developed the technique.

Disease Type	Mean (μg/ml)	Standard Deviation	Standard Error	Co-eff of Var.	Number
Simple Aneurysms	0.36	0.49	0.11	135.67	20
Inflamm. Aneurysms	0.77	0.67	0.21	87.21	10
Occlusive Disease	0.24	0.30	0.08	123.86	13

Comparison of the three groups by the Mann-Whitney test gives the following-

Inflammatory aneurysms vs. Simple aneurysms:
$U = 54$, $z = -2.0240$, $p = 0.043$

Inflammatory aneurysms vs. Occlusive disease:
$U = 26$

Simple aneurysms vs. Occlusive disease:
$U = 113$, $z = -0.6265$, $p = 0.530$

The complexity, expense, and time consuming nature of the assay for elastin fragments precluded the production of reproducibility figures for this data. Assays were carried out in triplicate, and the figure given for each patient is a mean of these three assays.

Because of this, and the small numbers investigated, the data and statistical tests of inter-group differences cannot be regarded as definitive. They should be regarded as data useful in the sense of a pilot study, and will be discussed later in this work.

Antitrypsin Phenotyping

Antitrypsin phenotyping was carried out using a standard isoelectric focussing technique as described above. 14 patients with inflammatory aneurysms, 36 patients with simple aneurysms, and 33 patients with aorto-iliac occlusive disease were tested. The results in terms of total numbers are shown below.

Antitrypsin Phenotypes.

Antitrypsin Phenotype	Inflammatory aneurysms	Simple aneurysms	Occlusive disease
MM	10	26	28
MS	2	4	2
MZ	1	4	2
FM	-	1	-
SS	1	1	-
ZZ	-	-	1
TOTAL	14	36	33

There are no clear cut differences in the frequency of inactive or partially active antitrypsin phenotypes between the three groups. Contingency tables were constructed for this data (see Below).

Contingency tables were made to compare simple with inflammatory aneurysms, and simple aneurysms with occlusive disease. Because of the small numbers in each of the groups other than MM phenotype, the table shown above was collapsed to include all phenotypes apart from MM in a separate group.

The small numbers involved in the construction of the second table demanded that Fishers Exact Test was used to calculate the probability of difference. In the third table, a Chi-squared test was used.

Base Table

Phenotype	Inflammatory	Simple	Occlusive
MM	10	26	28
Others	4	10	5

Simple vs. Inflammatory aneurysms.

Phenotype	Inflammatory	Simple
MM	10	26
Others	4	10

Fishers Exact Test: $P_1 = 0.271$
$P_2 = 0.283$

$\mathbf{P_{1+2} = 0.554}$

The Null Hypothesis is therefore accepted: There is no difference between the groups.

Simple aneurysms vs. Occlusive disease

Phenotype	Aneurysm	Occlusive
MM	26	28
Others	10	5

Chi-squared Test: $X^2 = 2.44$ (1 d.f. and Yates Correction)
$P > 0.5$

The null hypothesis is accepted: There is no difference between groups

Acute Phase Proteins.

These assays were performed on a calibrated immuno-turbidometer used routinely for clinical purposes. The techniques have been described above. The results for each acute phase protein will be dealt with in turn.

C-Reactive Protein.

Results of C-reactive protein assay.

Disease Type	Mean (mg/l)	Standard Deviation	Standard Error	Co-eff of Var	Number
Inflamm. Aneurysms	37.47	42.63	11.01	113.77	15
Simple Aneurysms	21.37	28.08	3.10	131.42	82
Occlusive Disease	18.05	20.61	3.39	114.17	37

Comparison of the three groups using the Mann-Whitney test gives the following results -

Inflammatory aneurysms vs. Simple aneurysms:
$U = 403$, $z = -2.2474$, $p = 0.025$

Inflammatory aneurysms vs. Occlusive disease:
$U = 163$, $z = -2.4542$, $p = 0.014$

Simple aneurysms vs. Occlusive disease:
$U = 1410$, $z = -0.6747$, $p = 0.499$

Alpha-1-Antitrypsin.

Results of Alpha-1-Antitrypsin Assay.

Disease Type	Mean (g/l)	Standard Deviation	Standard Error	Co-eff of Var	Number
Inflamm. aneurysms	2.31	0.5	0.13	21.89	15
Simple aneurysms	1.94	0.61	0.07	31.26	82
Occlusive disease	1.88	0.48	0.08	25.41	37

Comparison of the three groups using the Mann-Whitney test gives the following results -

Inflammatory aneurysms vs. Simple aneurysms:
$U = 383$, $z = -2.3191$, $p = 0.020$

Inflammatory aneurysms vs. Occlusive disease:
$U = 126$, $z = -3.0721$, $p = 0.002$

Simple aneurysms vs. Occlusive disease:
$U = 1420$, $z = -0.5582$, $p = 0.576$

Alpha-2-Macroglobulin.

Results of Alpha-2-Macroglobulin Assay.

Disease Type	Mean (g/l)	Standard Deviation	Standard Error	Co-eff of Var	Number
Inflamm. aneurysms	2.20	0.49	0.13	22.33	15
Simple aneurysms	2.47	0.77	0.09	31.23	82
Occlusive disease	2.46	0.74	0.12	29.91	37

Comparison of the three groups using the Mann-Whitney test gives the following results -

Inflammatory aneurysms vs. Simple aneurysms:
$U = 485$, $z = -1.2992$, $p = 0.194$

Inflammatory aneurysms vs. Occlusive disease:
$U = 226$, $z = -1.0429$, $p = 0.297$

Simple aneurysms vs. Occlusive disease:
$U = 1510$, $z = -0.0402$, $p = 0.965$

Caeruloplasmin.

Results of Caeruloplasmin Assay.

Disease Type	Mean (g/l)	Standard Deviation	Standard Error	Co-eff of Var	Number
Inflamm. aneurysms	0.45	0.10	0.02	21.24	15
Simple aneurysms	0.40	0.12	0.01	29.26	82
Occlusive disease	0.39	0.10	0.02	26.78	37

Comparison of the three groups using the Mann-Whitney test gives the following results -

Inflammatory aneurysms vs. Simple aneurysms:
$U = 414$, $z = -2.0067$, $p = 0.04$

Inflammatory aneurysms vs. Occlusive disease:
$U = 181$, $z = -1.9513$, $p = 0.05$

Simple aneurysms vs. Occlusive disease:
$U = 1471$, $z = -0.2643$, $p = 0.79$

Elastase Inhibitor Assay

This assay was originally given to us by the University of Sheffield. The original protocol has been used there in an experimental sense for some time and has been found to be highly accurate, reproducible, and to give excellent separation of phenotypes in alpha-1-antitrypsin assay. It is essentially an assay of the ability of human serum to inhibit the action of elastase upon a substrate molecule. In this case, the elastase used is highly purified porcine elastase, and the substrate SANA (see Methods section). For practical purposes over 90% of the antiprotease activity in human serum is due to alpha-1-antitrypsin, and the assay can be regarded as a functional assay of that molecule to all intents and purposes.

Inter-assay Reproducibility.

Details of the standardisation studies of this assay can be found in the Methods section above. Since reproducibility studies were performed in tandem with assays of patient samples, the results are given below.

Reproducibility was studied using the same technique as detailed above for the assay of Elastase-antitrypsin complex. In this case, 24 samples were rendered anonymous by a colleague and identified by number only. Each 'unknown' sample was assayed twice, in different assay batches, and the coefficient of variation calculated.

Reproducibility was also checked indirectly using the results of the three Sheffield standards included in each batch of patient assays as quality control. The results are given below.

Inter-Assay Reproducibility of Unknown Samples.

The results of the elastase inhibition assay for the 24 anonymous samples chosen for reproducibility studies are shown below.

Sample	Assay 1 (%)	Assay 2 (%)	Difference Squared
1	9	11	4
2	24.4	23.62	0.608
3	27.15	29.7	6.5
4	26.8	29.4	6.76
5	26.04	28.02	3.92
6	14.9	16.08	1.392
7	14	16.5	6.25
8	20.41	20.3	0.012
9	20.14	16.62	12.39
10	21.34	23.96	6.86
11	26.7	26.9	0.04
12	25.43	20.6	23.32
13	19.94	16.75	10.17
14	25.39	22.8	6.7
15	18.73	15.38	11.22
16	29.74	28.04	2.89
17	28.24	25.46	7.72
18	17.21	13.4	14.51
19	30.68	29.4	1.63
20	23.37	20.9	6.1
21	16.73	18.25	2.31
22	34.12	31.9	4.92
23	21.81	19.91	3.61
24	13.29	11.02	5.15

For the above data, the co-efficient of variation has been calculated using the same method as before.

Value mean ($\hat{x}_v$) - 21.90
Σ_{δ^2} - 148.98
n=48

Co-efficient of variation calculated as

$$CV = \frac{\sqrt{\frac{\Sigma_{\delta^2}}{n}}}{\hat{x}_v}$$

$$CV = \frac{\sqrt{\frac{148.98}{48}}}{21.90}$$

CV = 8.0%

Results of Assay of Standards.

Each figure represents residual elastase activity as a percentage of the total remaining after exposure to the sample serum.

Sample M (%)	Sample MZ (%)	Sample Z (%)
17	23	45
26.6	32.5	52.9
23.3	28.95	53.65
23.3	31.15	52.92
26.2	34	50.5
19.9	26.45	48.1

In the table above, Sample Z can be seen, as the least active phenotype of the three, to leave most residual elastase activity. In common with the reproducibility studies on unknown samples, the reproducibility of repeated assay of the Sheffield standards is good. The ability of the assay to separate phenotypes was also maintained.

Summarised results for the study patients are given below.

Elastase Inhibition Assay - Results of Assays of Patient Samples.

Disease Type	Mean (%)	Standard Deviation	Standard Error	Co-eff of Var.	Number
Inflamm. Aneurysms	13.40	3.79	0.98	28.30	15
Simple Aneurysms	21.35	10.25	1.25	48.00	67
Occlusive disease	19.47	12.46	2.17	64.02	33

Mean (%) refers to the mean percentage of residual elastase remaining after exposure to patients sample serum.

Comparison of the three groups using the Mann-Whitney test gives the following results:

Inflammatory aneurysms vs. Simple aneurysms:
$U = 196$, $z = -3.6763$, $p < 0.001$

Inflammatory aneurysms vs. Occlusive disease:
$U = 144$, $z = -2.3022$, $p = 0.021$

Simple aneurysms vs. Occlusive disease:
$U = 938$, $z = -1.2279$, $p = 0.219$

Radiology

Radiological investigations were carried out as detailed in 'Methods'. Each was scrutinised for its ability to detect the following characteristics, as detailed in 'Methods', above:

1. Presence of aneurysm
2. Upper extent of aneurysm related to the renal arteries
3. Origins of the renal arteries
4. Diagnostic features of inflammatory change

The study group comprised 79 patients in all, and included 15 patients with inflammatory aneurysms proven at operation and subsequent histopathological examination.

MRI was not available for use for some months following initiation of this work. Throughout the study, accessibility was limited by available time on the scanner. In some cases study patients did not undergo all 3 radiological investigations. For this reason, study group numbers are displayed as a Venn Diagram to clearly highlight the numbers of patients undergoing each combination of investigation.

Results for each modality are presented separately. Detailed comparisons are made between modalities later in this work.

This Venn Diagram (Figure 26) indicates the number of patients undergoing each combination of investigations. Total numbers of patients undergoing each investigation are therefore:

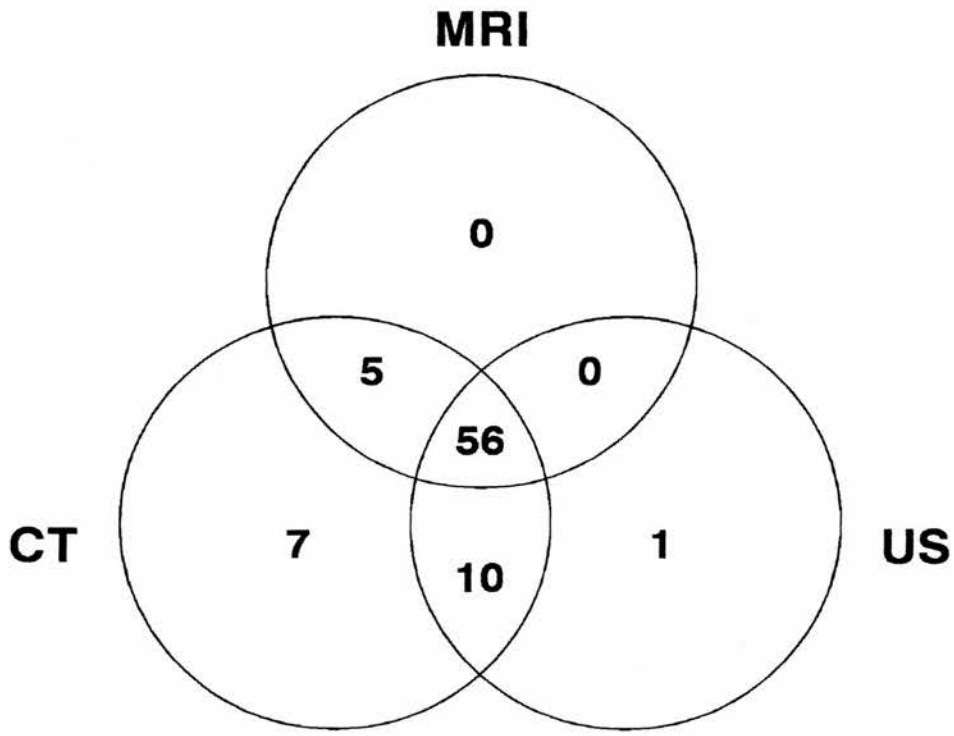
Ultrasound (US) - 67

Computed tomography (CT) - 78

Magnetic Resonance (MRI) - 61

Figure 29.

Venn diagram illustrating numbers of patients undergoing each radiological investigation.



56 patients underwent all 3 investigations. This included 15 patients with inflammatory aortic aneurysms. In all cases, radiology is correlated with results of inspection and histological investigation at and following operative treatment of the aneurysm. In the following 3 tables, numbers refer to the number of cases in which the stated characteristic was successfully detected.

Results for each Modality

1. Ultrasound.

Results of Ultrasound Examination in Determining Aneurysm Characteristics.

	Presence of Aneurysm	Level of Neck	Renal Artery Origins	Diagnosis of Type
Inflam. Aneurysm (n=15)	15	12	0	0
Simple Aneurysm (n=52)	52	34	0	52

It can be seen that ultrasound failed to differentiate simple from inflammatory aneurysms in all cases. It also failed to visualise the origin of the renal arteries in all cases. In all cases where a statement of the level of the aneurysm neck was made, this inference was made either from the aneurysms position in relation to other intra-abdominal organs, or, most commonly, from visualisation of the superior mesenteric artery origin. In 3 cases of inflammatory aneurysm, the level of the aneurysm neck as estimated by ultrasound was unavailable or incorrect. In two cases, this was due to

overlying bowel gas. In the third case, the level was unclear, but appeared to involve the renal arteries on ultrasound. It was clearly infrarenal at operation.

In the simple aneurysm group, 18 patients had incorrect or unavailable estimation of the level of the aneurysm. The commonest cause for lack of definition of the upper level of the aneurysm was that it simply could not be clearly seen on realtime ultrasound examination. This accounted for 8 cases. In five cases, the upper end of the aneurysm was obscured by gas, and visualisation of the entire aortic aneurysm was impaired. In two cases, the level of the aneurysm neck was wrongly stated as being above the level of the renal artery origins, and in two cases wrongly stated as being below. In one patient his stocky bodily habitus made estimation of the level of the neck impossible.

In summary, ultrasound was useful in the preliminary detection of aneurysmal change in the abdominal aorta and in the initial estimation of the level of the neck of the aneurysm in relation to the level of the renal arteries. It failed in the detection of inflammatory change and in visualising the origins of the renal arteries.

2. Computerised Tomography.

Results of Contrast Enhanced Computerised Tomography In Determining Aneurysm Characteristics.

	Presence of Aneurysm	Level of Neck	Renal Artery Origins	Diagnosis of Type
Inflam. Aneurysm (16)	16	11	2	7
Simple Aneurysm (62)	62	56	8	53(3)

As expected, C.T. succeeded in determining the presence of aneurysmal change in all cases. It offered improved detection of the level of the neck of the aneurysm, diagnosis of type, and visualisation of the origins of the renal arteries.

Errors in estimation of the level of the aneurysm neck were made in 5 cases of inflammatory aneurysm. In 3 cases, the upper end of the aneurysm was simply unclear or not seen on CT, while in 2 cases, the neck was incorrectly stated as lying above the level of the renal arteries. In the 6 cases of simple aneurysms where CT failed in this respect, 2 cases were due to lack of clarity, and 4 were wrongly called supra or juxta renal.

C.T. failed to show the origin of the renal arteries in most cases. Successful visualisation was largely by chance, and the likely reasons for this are discussed below.

Diagnosis of aneurysm type, and the recognition of 'typical' features of inflammatory change by C.T. was moderately accurate. 7 cases of inflammatory aneurysm were correctly diagnosed by C.T. by the presence of contrast-enhancing anterior soft tissue masses and distortion of surrounding anatomy. In the group of simple aneurysms, results were less good. In 6 cases, C.T. incorrectly showed features compatible with inflammatory change, and were therefore false positives. In a further 3 cases, leakage of the aneurysm was suggested when none was present at subsequent operation. These 3 cases are included in parentheses in the table above.

In summary, as expected, C.T. made the diagnosis of aneurysm in all cases, but was disappointing in its ability to assess the level of the aneurysm neck, and to image the renal artery origins. It was moderately accurate in making the diagnosis of inflammatory change, but there were a disturbing number of false positives in the simple aneurysm group.

3. Magnetic Resonance Imaging.

Results of Magnetic Resonance Imaging in Determining Aneurysm Characteristics.

	Presence of Aneurysm	Level of Neck	Renal Artery Origins	Diagnosis of Type
Inflam. Aneurysm (15)	15	15	15	15
Simple Aneurysm (46)	46	46	44	46

Magnetic Resonance Imaging was as successful in the diagnosis of aneurysmal change as the other two modalities. It was accurate in the assessment of neck level and imaging the renal artery origins. There were two failures in the imaging of the renal artery origins in the simple aneurysm group. In one case, the renal arteries could not be found with transverse, saggital or coronal scans. In the other case, the patient, initially fearful of the enclosed nature of the M.R.I. scanning apparatus, was able to tolerate only initial pilot scans before asking to stop the examination. This was the only failure of the M.R.I. scanning programme on the grounds of claustrophobia, but even pilot scans were able to detect the presence and level of the aneurysm.

Figure 30.

Renal artery visualisation using MRI. The figure shows a transverse abdominal scan above the level of the aneurysm. The renal arteries are labelled.

It became apparent during the initial experience of M.R. imaging that there were specific differences in the appearance of the wall of inflammatory aneurysms compared with the appearance of simple aneurysms. Following acquisition of the M.R. scans on the first four patients with inflammatory aneurysms, it was noted that the inflammatory aneurysms appeared as arrays of concentric alternating layers of high and low signal intensity, best seen on the T1 weighted images and STIR sequences.

Comparison with the scans of simple aneurysms obtained to that date suggested that the presence of three or more high signal intensity layers external to the vessel lumen was highly suggestive of inflammatory change, whereas fewer layers indicated a simple origin to the aneurysm, (Figure 31 b+c). Using these features as diagnostic criteria, the remaining patients in the study series were scanned, and a pre-operative diagnosis declared in all cases, as in studies of the other two modalities.

Assessment of aneurysm type in the single case with claustrophobia was made on the basis of spin-echo sequences rather than the images found to be most accurate i.e. STIR or T1 weighted.

Figure 31a.

Inflammatory aneurysm seen on transverse abdominal CT. The rind of inflammatory tissue enhances on contrast injection. In this case, the inflammatory tissue (arrow) encases the inferior vena cava (V).

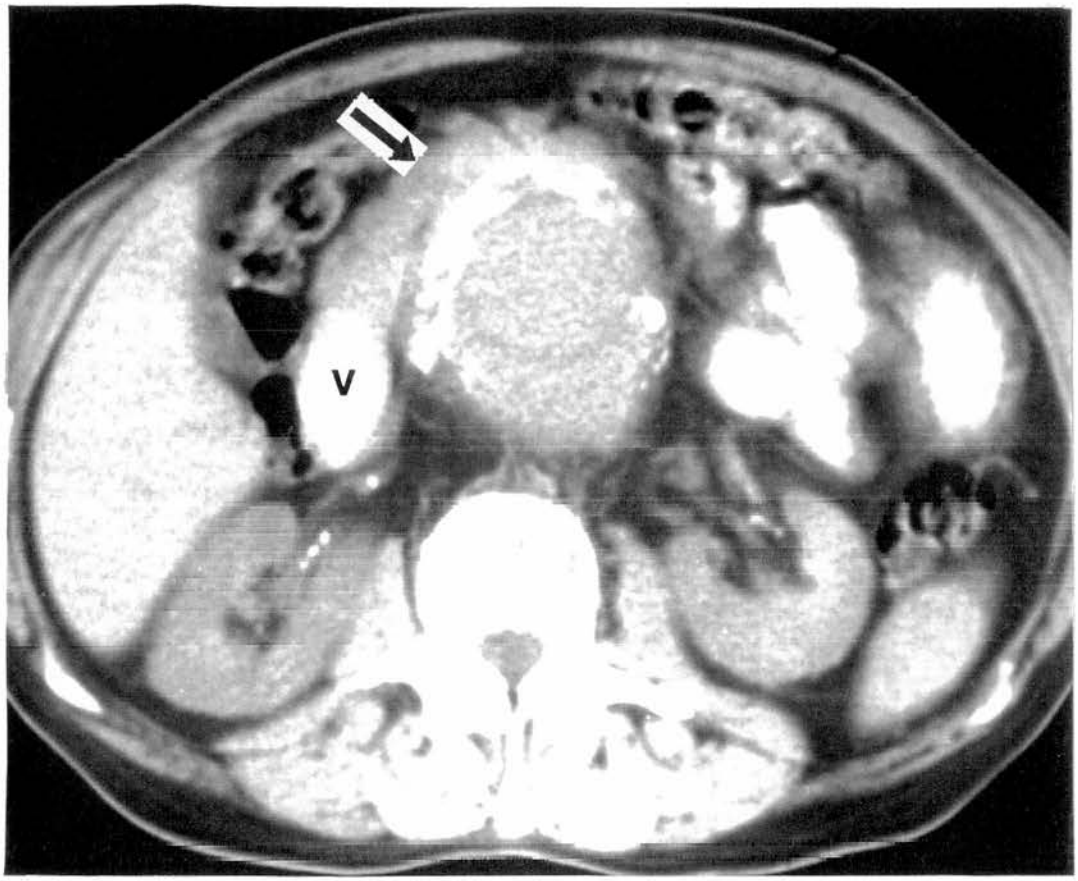


Figure 31b.

Inflammatory aneurysm on transverse abdominal MRI scanning using the STIR sequence. Inflammatory tissue is displayed as concentric rings of high signal density (arrow).

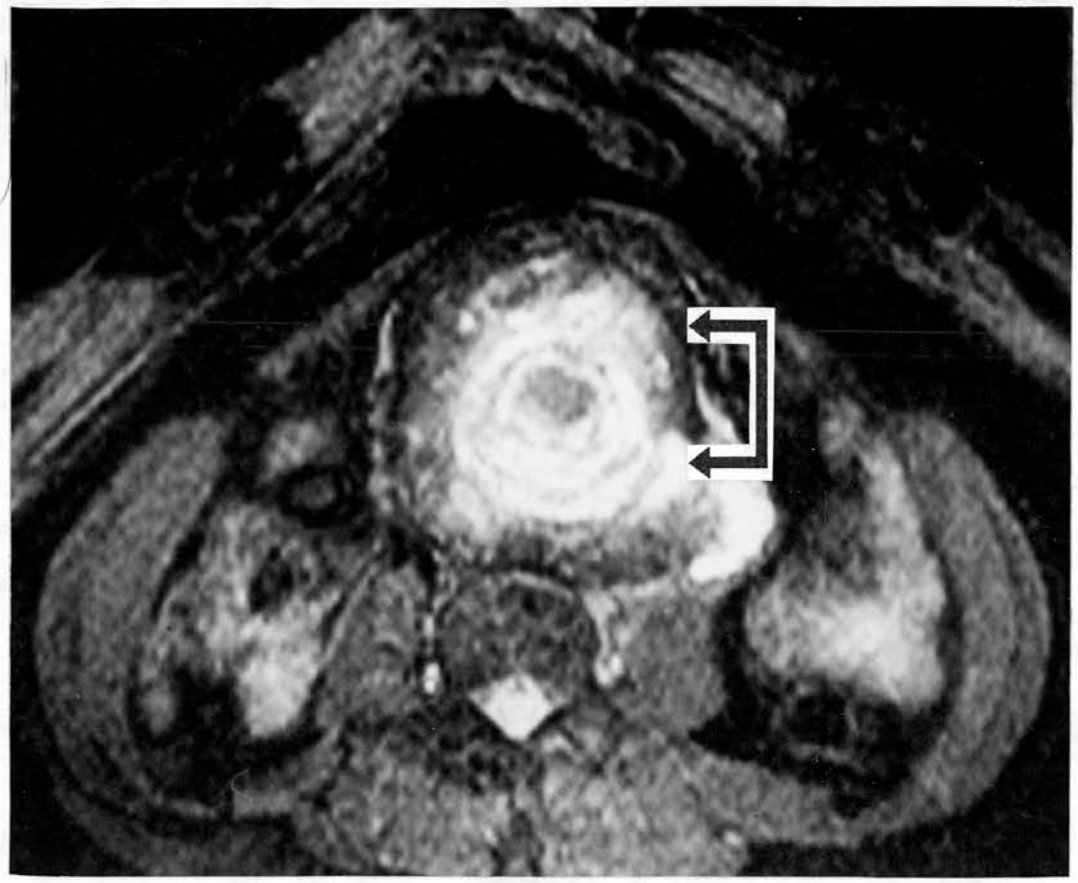
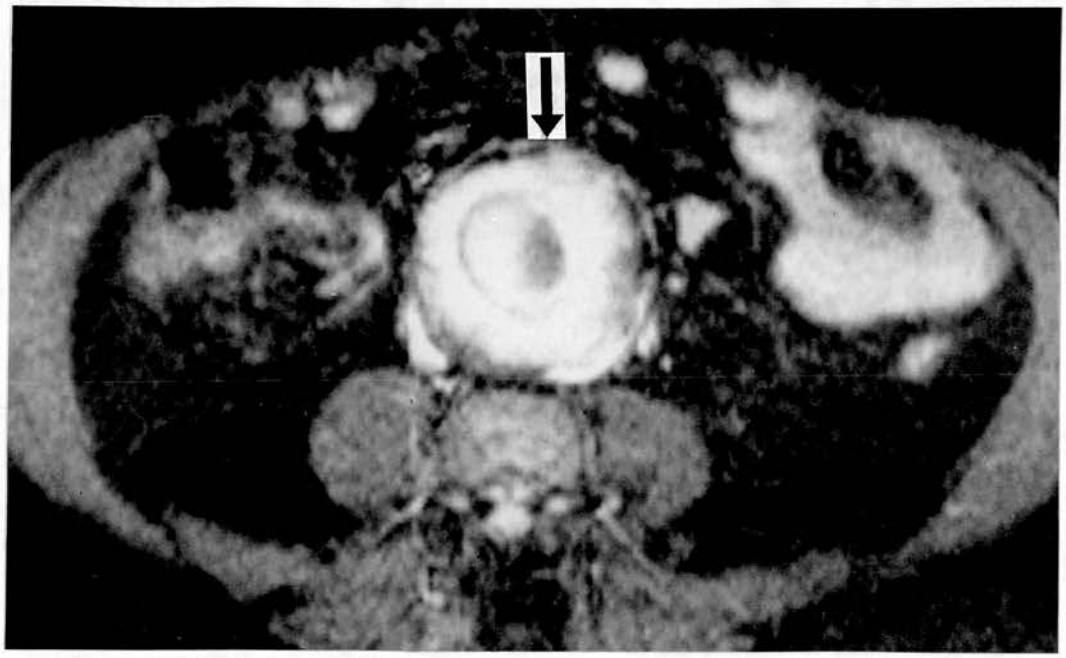


Figure 31c.

Simple (non-inflammatory) aneurysm on transverse abdominal MRI scanning using the STIR sequence. There is no evidence of the complex multi-layering in this case. The aorta is indicated (arrow).



In addition to this new diagnostic finding, M.R.I. was useful in the demonstration of a number of special features in selected cases. These are illustrated below.

Figure 32.

Saggital MRI scan of a simple thoracic aneurysm, showing the unique view of a posterior 'blow-out' (arrow). At operation, the posterior wall of the aneurysm was found to be deficient at this point.

Figure 33a.

A horseshoe kidney (arrow) in a case of aortic aneurysm. Detailed study of the MR images revealed that this aneurysm has a complex multilayered wall structure suggesting inflammatory change. This was confirmed at operation.

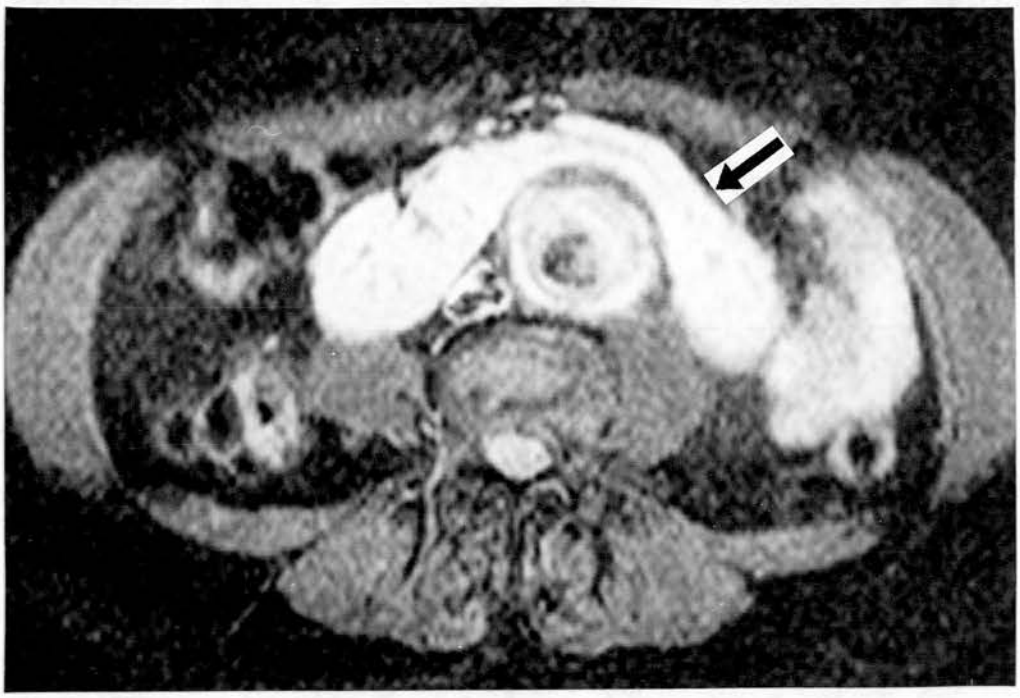
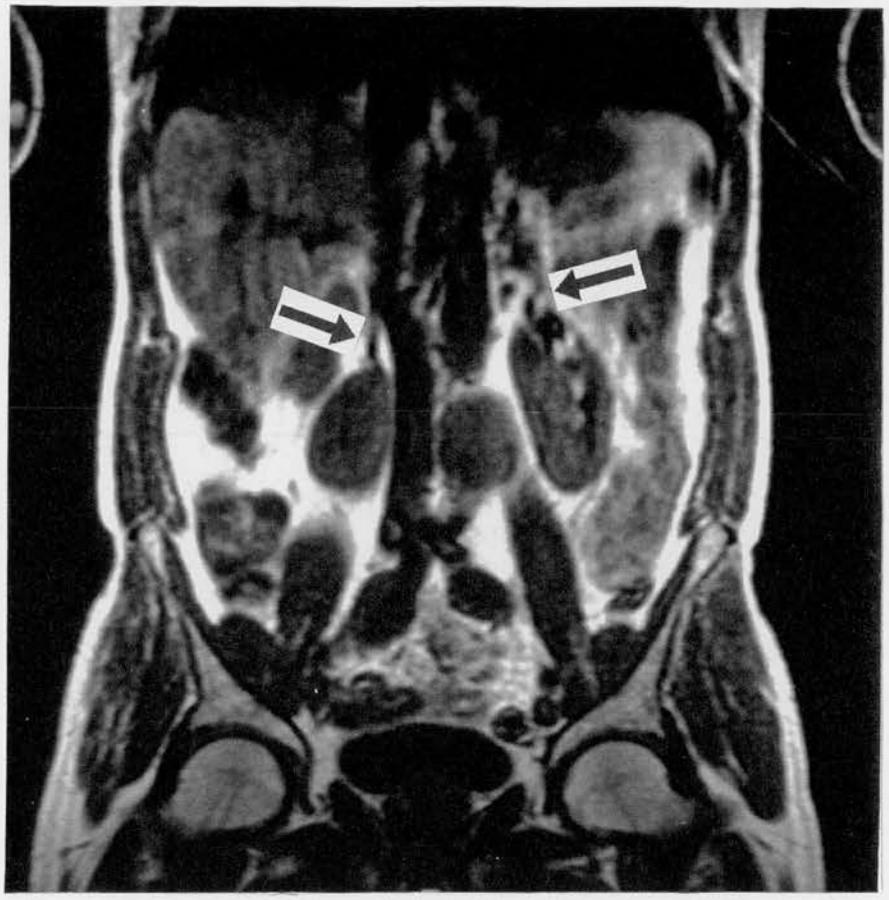


Figure 33b.

Renal artery visualisation using MRI in coronal section of the same patient as Fig 30a. The positions of the renal arteries are indicated (arrows).



In Vitro M.R.I. Studies

The laminated appearance of inflammatory aneurysms was distinct, but the reason for it obscure. In an effort to determine the reason for it, 4 specimens of aneurysm wall obtained at operation were subjected to M.R.I. imaging. These were compared to images obtained of 4 specimens of simple, or non-inflammatory, aneurysm. The technique was as detailed in Methods above.

Figure 34a.

In vitro MR images of wall samples from inflammatory aneurysms. The samples (arrowed) are immersed in water, and supported on water saturated foam rubber. The wall appears layered.

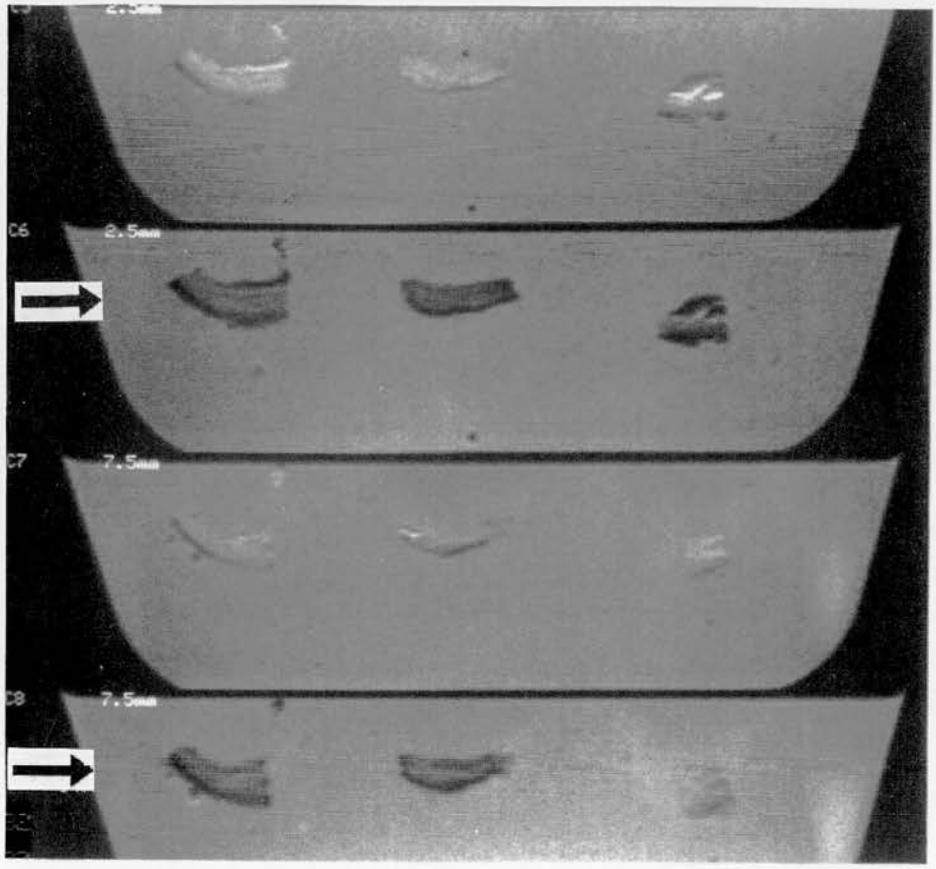
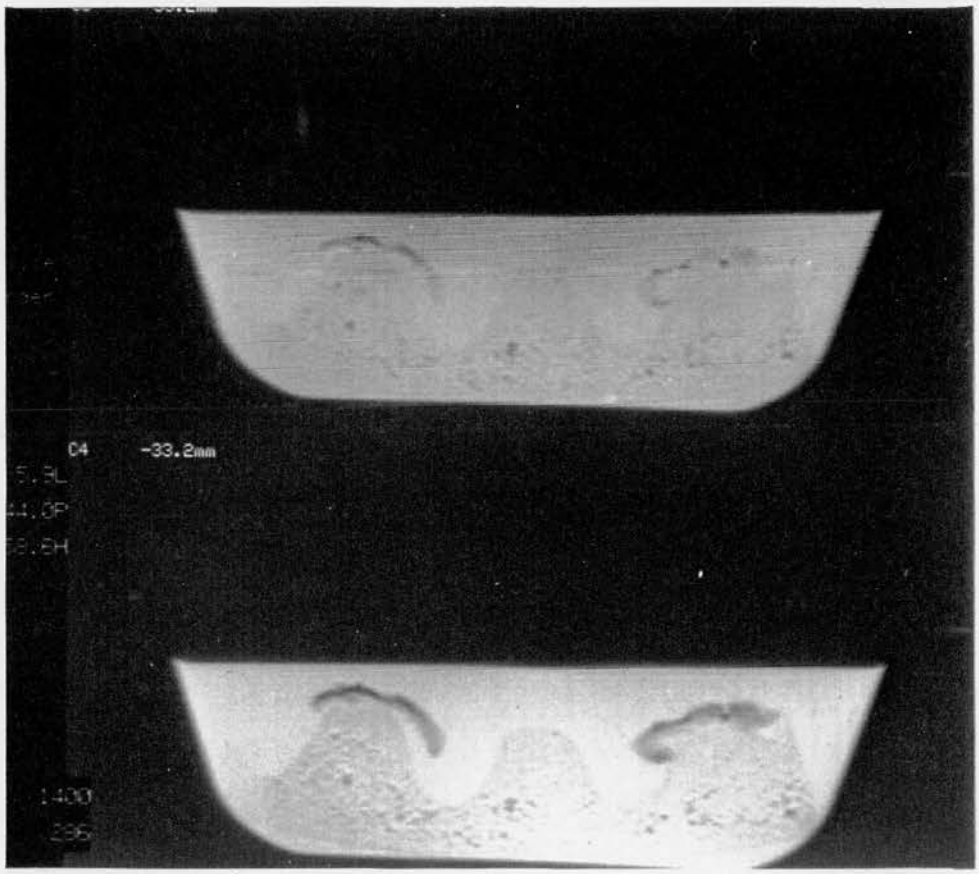


Figure 34b.

Similar images of wall samples from non-inflammatory aneurysms. No evidence of wall complexity or layering is seen.



The inflammatory specimens appear laminated. Bearing in mind that thrombus was removed from the lumenal surface of these specimens during preparation, factors in the fibrous wall itself must be responsible for this appearance.

Histology.

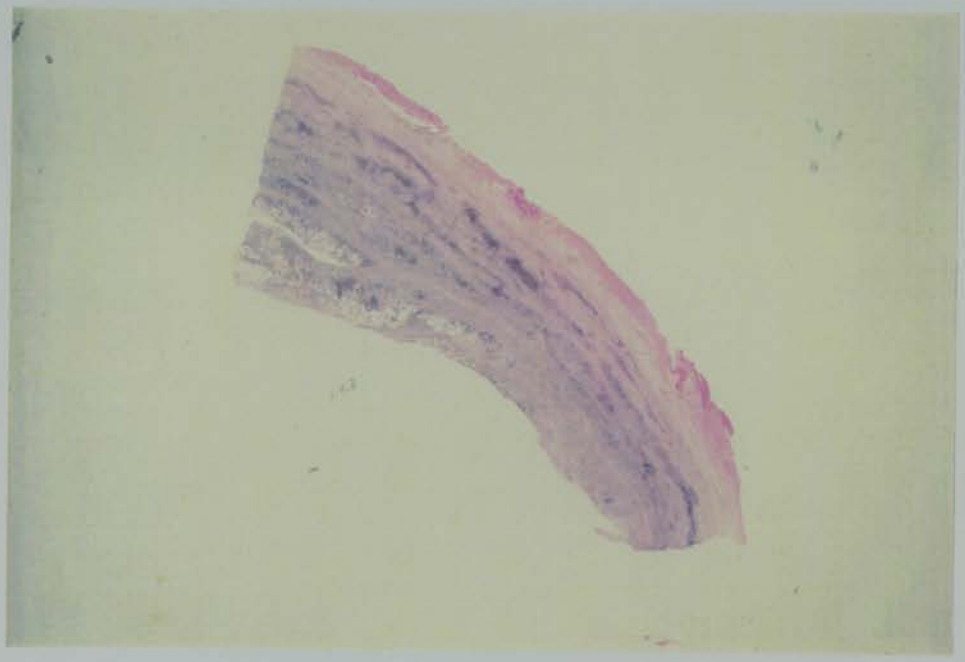
Slides for histological examination were made from each of the four specimens of inflammatory tissue, and examined. The results are shown below.

Figure 35a.

Histology slides of inflammatory aneurysm wall (H&E). These are the same specimens as shown in Fig 30. Once again, there is a layered appearance, broadly corresponding to layers of lymphocytic infiltrate and fibrous tissue.

Figure 35b.

Another specimen of aortic wall prepared as in Figure 32a above, and showing the same features of layering.



SUMMARY.

15 patients with inflammatory aneurysms, 83 patients with simple aneurysms and 37 patients with severe aorto-iliac occlusive disease are included in the prospective studies.

Diagnosis of inflammatory aneurysm is impossible on the basis of risk factor analysis, clinical examination, or haematological investigation. Raised plasma viscosity, pain and weight loss occur more frequently in association with inflammatory aneurysm than simple aneurysm, but the differences are not sufficient to be diagnostic.

Prospective findings on the operative appearances of inflammatory aneurysms, operative technique required and graft type confirm the results of the retrospective study. The 30 day mortality for operative treatment of inflammatory aneurysms was improved considerably.

Detailed biochemical studies of the peripheral blood of all three groups of patients revealed increases in the levels of neutrophil elastase, elastin fragments, acute phase proteins, and total antielastase activity in the group with inflammatory aneurysms. The distribution of alpha-1-antitrypsin phenotypes did not differ between the groups.

The investigation of Magnetic Resonance Imaging in aneurysms disease has resulted in improved definition of the physical extent and relationships of both simple and inflammatory aneurysms. In addition, unique diagnostic appearances of inflammatory aneurysm wall have been described.

The clinical and pathological significance of these results will be discussed in the following chapter.

CHAPTER 9

DISCUSSION

INTRODUCTION.

This discussion will consider inflammatory aortic aneurysms under three main headings: Current problems, Diagnosis/treatment, and Aetiology.

Inflammatory Aneurysms - Current Problems.

Inflammatory aortic aneurysms represent an interesting but under-researched aspect of surgery and are recognised as a distinct entity both clinically and pathologically. To date however, much research effort has been directed at their histological characteristics and the technical aspects of their repair rather than questions of ætiology or pathogenesis.

Currently, radiological diagnosis of inflammatory aneurysm uses computerised tomography (CT) as the 'gold standard'. Most major series in the literature, however, suggest that diagnostic accuracy is poor, making the correct diagnosis in approximately 50% of cases.[195,241] No information is available to date on the use of more recently introduced modalities such as magnetic resonance imaging (MRI).

It is apparent from major studies that the presence of an unsuspected inflammatory aneurysm at operation may lead to abandonment of the procedure, or a marked increase in operative mortality.[193,196]

New information from the retrospective series included in this work, which has not previously been apparent suggests that operations performed for inflammatory aneurysm disease take longer and require more blood and colloid infusion. Major intra-operative changes in technique may also be required when the presence and extent of the inflammatory change is not apparent pre-operatively.

Operative (30 day) mortality for inflammatory aneurysms was higher than that for simple aneurysms in this retrospective study, the principle cause of death being myocardial infarction. Even outwith the 30 day period, post-operative survival remained worse in the inflammatory group.

These findings confirm that operations on inflammatory aneurysms are more difficult and dangerous than those for simple aneurysms. It is therefore in the best interest of both patient and surgeon that the diagnosis of inflammatory change should be made accurately pre-operatively. With a reliable diagnosis, an appropriate operative approach can be planned. Facilities can also be made available for the increased demands on intra- and post-operative care required, and for more detailed long term follow-up.

A number of proposals have been made linking biochemical changes with the formation of both simple and inflammatory aneurysms, and these are outlined in Chapters 2 & 3. Despite this, little is known about the metabolic activity of inflammatory aneurysm, its relationship to pathogenesis or its effect on patients. If more is known of its precise nature, then more sensitive diagnostic techniques and more specific forms of non-operative treatment may be possible, either prior to surgery, or instead of it.

The biochemical and hæmatological investigations included in the prospective part of this work were designed to examine some of the questions surrounding the nature of the inflammatory reaction in inflammatory aneurysm disease.

Diagnosis and Treatment.

A number of major studies of inflammatory aneurysms have commented on the clinical and haematological and radiological features associated with the presence of inflammatory aneurysms.[102,193,195,207] None have been successful in identifying any characteristic sensitive enough to be used in diagnosis.

In this study, a five year retrospective series was used to highlight difficulties in diagnosis, operative management and outcome of inflammatory aneurysms, and to compare the results with a similar series of simple aneurysms.

There were no patient characteristics in terms of age, sex, symptoms or concomitant disease which were reliable enough to be used as screening or diagnostic criteria for inflammatory aneurysms. Systemic illness in the presence of a palpable aortic aneurysm more strongly suggests the diagnosis of inflammatory aneurysm. These systemic symptoms, however, are non-specific and, being present in a minority of inflammatory aneurysms, are insufficient to make a certain diagnosis in the absence of any objective evidence.

The easiest and cheapest useful hæmatological technique was plasma viscosity or E.S.R. This in itself was not sufficient to make the diagnosis, being a non-specific indicator of inflammation but was enough to raise suspicion, and prompt further radiological investigation in many cases.

Unfortunately, both ultrasound and contrast-enhanced CT have proved disappointing in their ability to provide objective evidence. They missed almost half of subsequently confirmed cases of inflammatory aneurysm

in the retrospective series. Both this and the insensitivity of hæmatological techniques resulted in many aneurysms proceeding to operation without the diagnosis of inflammatory change being made.

In respect of presentation, clinical features, and diagnostic accuracy, this retrospective study simply confirmed the findings of the largest clinical and radiological studies available from the literature.

In the prospective part of this work, three radiological modalities were assessed for their sensitivity in the detection of the physical characteristics of aneurysms and the diagnosis of inflammatory change. It was considered likely that because of its enhanced ability to display differences in soft tissue characteristics, the use of Magnetic Resonance Imaging would improve the pre-operative diagnosis of inflammatory change in aortic aneurysms.

While it was not anticipated that detection of specific features of inflammation would be good, ultrasound failed to detect any of the inflammatory aneurysms in this series. It was, however, moderately successful in the diagnosis of the level of the aneurysm neck, albeit by indirect assessment rather than by visualisation of the level of the renal artery origins.

Computerised tomography was only as good as ultrasound in assessment of the level of the aneurysm, and this was somewhat surprising. It may be because, while ultrasound is performed in realtime, is to some extent interactive, and is able to image in different planes, C.T. is limited practically to transverse imaging. Image plane is then affected by patient positioning and, because of the inability of this modality to image in realtime, the usefulness of a change of position of the patient can only be assessed after a further scan.

It is for the same reason that imaging the origins of the renal arteries was inadequate using CT. The renal arteries were only seen on a few scans where the scan plane happened to include them. While it is possible to image the renal artery origins using CT, this involves the use of more scan time, more radiation for the patient because of repeat scans, and repeated contrast injection. In the context in which visualisation of the renal arteries is specifically required, large doses of contrast material may compromise renal function.

C.T. wrongly estimated the level of the aneurysm neck to be above or to include the renal arteries in 6 cases (2 inflammatory aneurysms and 4 simple aneurysms). Had only C.T. evidence been available, then either the operative approach would have been more aggressive, or (more likely), the patients would have been exposed to further contrast material and radiation in the form of angiography, a procedure not without morbidity and mortality.

The number of false positive examinations both for evidence of leaking aneurysms and inflammatory change is alarming. It is likely to be related to the inability of CT to distinguish the nature of soft tissue masses with sufficient detail. Enhancing masses of soft tissue anterior of lateral to the aneurysm were seen in all cases of false positive CT scans. None of these were apparent on MRI, which showed simple wall structures in all cases.

Magnetic Resonance Imaging (MRI) excelled in the detection of the level of the aneurysm, the origin of the renal arteries, and detection of specific features of inflammation. Direct visualisation of the origin of the renal arteries is of use in determining the level of the aneurysm neck and its relationship to the renal arteries with accuracy.

Limited information is also available from the MRI scan about the state of patency of the renal arteries, as flowing blood leaves a so-called flow

void on MRI scanning, appearing as a low signal or black area.

The most prominent finding of this study of the use of MRI in inflammatory aneurysm disease was the ability of MRI to detect specific features of inflammatory change. The multi-layered 'onion-skin' appearance produced using MRI scanning was characteristic, and highly sensitive in detecting inflammatory tissue.

STIR sequences as used in the imaging for this study are most sensitive at showing differences in proton density, essentially water content, in tissue. That it should produce such detailed images of the wall of aneurysms was something that was not expected. Although preliminary evidence shows that the layering on these MRI images correspond to histological layering seen on light microscopy, further work will need to be done in this regard.

In cases in which the diagnosis of inflammation was made on MRI but not on CT, MRI made the correct diagnosis in all cases using the criteria of complex wall structure. In 3 cases, it also showed soft tissue masses anterior or lateral to the aorta not shown on contrast enhanced CT.

MRI displays all the information necessary for the diagnosis and 'staging' of both simple and inflammatory aneurysm disease without the use or necessity of injected contrast material or ionising radiation. In addition, the coronal and saggital images are most useful in displaying the exact morphology of an extensive aneurysm in an easily assimilated form.

There are limitations to the use of MRI for the assessment of aneurysm disease. Currently it cannot be used in the emergency situation in the anæsthetised patient because of the presence of metallic parts on the anæsthetic machine. Care must be taken in patients with cardiac pacemakers and other metallic implants, although with appropriate precautions there is no

contra-indication to scanning these patients.

In this study the only patient who could not be scanned was claustrophobic.

All patients in both the retrospective and prospective arms of this work were treated operatively, and had no adjunctive treatment. Treatment with corticosteroids remains the only active alternative to operation, but the response is uncertain, and the results anecdotal. There are theoretical dangers in the use of steroids, and no major series has been reported dealing with their use.[203]

Operative treatment of inflammatory aneurysms in the retrospective series was associated with a higher operative mortality. The prognosis was improved in the prospective group. Although this did not appear to be due to any reduction in blood loss transfusion requirements or operation time, the availability of detailed radiological investigation and the reliable diagnosis of inflammatory change allowed planning of the operative approach. Improvements in prognosis may also have been due to changes made in anaesthetic techniques in the light of the possible diagnosis, which were not assessed in this study.

Aetiology of Inflammatory Aneurysms.

There is histological evidence of inflammation within the wall of inflammatory aneurysms. The cellular picture is of 'chronic' inflammation, with an abundance of lymphocytes, plasma cells and macrophages.[102,106,195,197] The progressive nature of inflammation and its failure to resolve without treatment suggest that the fibrous inflammatory response is active, rather than

the 'burnt out' and passive result of a short-term insult. The cause of the inflammation is questionable, but together with the generally accepted theory that aneurysm leads to fibrosis, the research of Parums and Mitchinson [197,201] suggests that inflammation is due to expression of an antigen through severely damaged medial tissue. This is certainly possible, and might be expected to lead to the type of response seen in inflammatory aneurysms - that is the active production of fibrous tissue.

Loss of medial elastin is a prominent feature of inflammatory aneurysm. This could be a physical effect of aortic dilation, or due to continuing active destruction by elastases. Increased active elastin destruction could be due to an absolute increase in elastases, or a decrease in elastase inhibition. Powell et al. have found increased levels of neutrophil elastase in inflammatory aneurysm wall, and assay of this enzymes inactive complex in the peripheral blood has been used clinically in monitoring the activity of both rheumatoid arthritis and inflammatory bowel disease.[162,266,267] Cannon and Read have attributed aortic aneurysm formation to a defect in elastase inhibitory mechanisms, and both they and other authors have provided parallel models of elastin destruction in the lungs of smokers.[149-152]

The biochemical investigations in the prospective part of this work were chosen to investigate -

1. Evidence for a systemic response to inflammation in patients with inflammatory aneurysms.
2. Differences in some aspects of peripheral blood biochemistry in patients with inflammatory aneurysms indicating differences in elastin metabolism compared to patients with simple aneurysms.

Simple aneurysms represent a degenerative disease. This is to some extent borne out by the results of the biochemical investigations in the prospectively studied series, as levels of acute phase proteins in the peripheral blood of patients with simple aneurysms approximate to those seen in severe aorto-iliac occlusive disease. On the other hand, there is evidence to suggest that active inflammation is present in patients with inflammatory disease, with raised levels of the acute phase proteins c-reactive protein, caeruloplasmin, and alpha-1-antitrypsin. It therefore seems likely that inflammatory aneurysms represent an active and ongoing disease process, rather than the inactive end stage of some previous insult.

One of the acute phase proteins, alpha-1-antitrypsin (a-1-at) is discussed in more detail below, because of its role in the metabolism of elastin.

As the principal inhibitor of elastase in the peripheral blood, a-1-at has been implicated in the ætiology of simple aneurysms. The mechanism which has been proposed is that deficiencies in antitrypsin levels or function have allowed increased, uninhibited action of elastase in the aortic wall, leading to aneurysm formation.[149-152]

The work of Parums & Mitchinson suggests that inflammatory aneurysm represents an extreme form of degenerative aneurysm in which the aortic medial coat is excessively thinned. If the mechanism linking defective antitrypsin with degeneration were to hold, the extreme degeneration of inflammatory aneurysms might be associated with a more marked defect in antitrypsin function.

That some defect in antitrypsin is responsible for the formation of simple aneurysms seems untenable in the light of the results of this study, as the total assay, phenotypes, and activity of a-1-at are no different in patients

with simple aneurysms than severe aorto-iliac occlusive disease.

Also contrary to the mechanisms proposed above, alpha-1-antitrypsin levels are higher in established inflammatory disease than in the other two groups. Again, there is no excess of inactive phenotypes in the inflammatory group, and the increase in antitrypsin levels is accompanied by an increase in antiprotease activity.

This is simply the non-specific 'damage limitation' action of a-1-at in its role as an acute phase protein, and occurring as a response to inflammation. It cannot be regarded in any way as specific to inflammatory aneurysm.

Even though this study is of patients with established disease, and the rise in alpha-1-antitrypsin is nonspecific, it is possible to hypothesise from the results presented that defective antiprotease function does not *predispose* to inflammatory aneurysm. If it were to, some abnormality in the acute phase response or antiprotease function might be expected to be present. No evidence has been found to support this.

Assay of neutrophil elastase complex in this study has suggested that levels are increased in the peripheral blood of patients with inflammatory aneurysm disease. This is likely to reflect increased production in the wall of the inflammatory aneurysm.

Some confirmation that the elastase increase detectable in the peripheral blood as complex with alpha-1-antitrypsin is resulting in increased elastin destruction is provided by the finding of increased levels of elastin fragments in the peripheral blood. Although the study of elastin fragments in this work was limited by small numbers, it provides some support for the proposal that there is increased and active mural elastin destruction in inflammatory aneurysms.

The finding of an association between neutrophil elastase and inflammatory aneurysms is curious, as in general, neutrophils are not a prominent feature of the cellular response in the wall of inflammatory aneurysms.

Unpublished data from Hornebecke et al in France suggest that this is not necessarily a contradiction, and that macrophages may elaborate elastases antigenically indistinguishable from that elaborated by polymorph neutrophils. Macrophages are a feature of chronic inflammation, and of the walls of inflammatory aneurysms.

Elastin is a major constituent of vascular media, and evidence has been presented to support its active destruction. As elastin is destroyed, and medial destruction proceeds, it is conceivable that more antigen is released, and that medial inflammation increases as a result. We are therefore left to speculate that a disease, initially degenerative (Simple aneurysm) provokes an inflammatory response, which through destructive inflammation serves to self perpetuate.

The following mechanism is suggested for the pathogenesis of inflammatory aneurysm. Findings from this study which support each step are given in bold type:

1. Medial damage in simple aneurysm leading to extrusion of unknown antigen - likely to be ceroid.

2. Antigen-antibody interaction. Chronic active inflammation in established disease.

3. Inflammatory reaction with release of elastase and tissue destruction involving elastin. **(Elastase/antitrypsin and elastin fragments increased in peripheral blood)**

4. Non-specific response to inflammation - release of acute phase proteins. Normal, non-specific rise in elastase inhibitory activity.
(Increased acute phase proteins, increased assay of phenotypically normal a-1-at, and increased functional activity of a-1-at)

5. Ongoing destruction of elastin releases more antigen, and cycle continues.

As inflammation is a gradual and continuous process, there is likely to be an intermediate clinicopathological type of aneurysm which is neither simple or established as a 'typical' inflammatory aneurysm.

It is occasionally found that, rather than a full blown inflammatory response with hard fibrous plaques and ureteric indrawing, there is nonetheless peripheral organ adherence to the aneurysm. The duodenum may be 'stuck' to the aneurysm wall by easily separable adhesions rather than densely adherent and included in a block of fibrous tissue. The wall is somewhat thinner than established inflammatory aneurysm, and lacks the white 'sugar icing' appearance.

It is likely that this represents the intermediate stage in the pathogenesis of inflammatory aneurysm, which without treatment will progress to display the characteristic dense fibrosis and organ involvement.

Summary.

In view of the failure of clinical, biochemical and haematological investigations to be sufficiently sensitive to serve as diagnostic tests for inflammatory aneurysm, it is likely that future developments in the diagnosis of inflammatory aneurysm will be the province of the radiologist. Radiological investigations carried out for this study have revealed a number of

shortcomings in the techniques regarded to date as 'Gold Standard' investigations for both simple and inflammatory aneurysms. MRI has been shown to be highly successful in the detection of inflammatory change, and in displaying the physical characteristics of both simple and inflammatory aneurysms. Its use has resulted in improved operative planning and a reduction in operative mortality for inflammatory disease.

MRI is currently far from freely available, and is relatively expensive when compared to CT scanning. It is currently in the same position as was CT in the mid 1970s, when scanners were scarce and expensive, and its abilities and limitations under scrutiny. It is almost certain that because of its detailed and completely non-invasive imaging abilities, MRI will become at least as commonly available in the future. Already it has the ability to provide all of the information required in aneurysm disease. With technological advances already under way, it is probable that MRI will be used even more extensively to provide even more information, including flow information from the aneurysm and the distal circulation.

Detailed investigation of peripheral blood biochemistry in patients with aortic aneurysm disease suggests that inflammatory aneurysms are the site of active inflammation with elastin destruction. These appear not to be due to any generalised defect in elastase inhibition, but rather a consequence of mural inflammation. These findings have reinforced some current theories of the aetiology of inflammatory aneurysms, such as that of Parums and Mitchinson and have led to speculation on a self-perpetuating mechanism in their pathogenesis.

There is already some experimental evidence that aneurysm disease may be altered by treatment with drugs other than steroids. Much of

this work is due to a few workers in large centres (ie David Tilson).[264] As knowledge of the mechanisms of the inflammatory process accumulates, it may be possible to arrest the destruction of aneurysm wall tissue in the future using pharmacological means.

CHAPTER 10

CONCLUSIONS

From the work presented above, the following conclusions are drawn.

1. Inflammatory aneurysms remain moderately common. They may present complex problems in operative management, and may be associated with increased operative mortality.

2. Although patients with inflammatory aneurysms display certain symptoms and hæmatological abnormalities more commonly than patients with simple aneurysms, none of these are sufficiently sensitive or specific to make the diagnosis with certainty.

3. The surgical treatment of inflammatory aneurysms may demand considerable changes in operative technique. Operative treatment also requires more time and blood/colloid transfusion. It is accompanied by a poorer short term prognosis than for simple aneurysm disease. This increases the importance of accurate pre-operative diagnosis.

4. Use of commonly available radiological techniques such as ultrasound and computerised tomography is not sufficiently sensitive or specific for clinical purposes. Reliance on C.T. features is potentially dangerous if decisions on operative approach are made on that basis alone. C.T. may misdiagnose the level of the neck of aneurysms with regard to the renal arteries, and in this respect also, even angiography is not completely accurate.

5. Magnetic Resonance Imaging represents a new modality for investigation of aortic aneurysm disease of all types. Its multiplanar imaging capability is useful in gaining a more complete understanding of the extent of complex aneurysms, especially those associated with other abnormalities or extending into the thorax.

M.R.I. is particularly accurate in demonstrating the level of the aneurysm neck in relation to the renal artery origins.

The complex wall structure shown on inversion recovery M.R.I. images is both sensitive and specific in the detection of inflammatory aneurysms. The laminated appearance, seen also in in-vitro scans appears to correspond in general terms with light microscopic findings.

6. The ætiology of inflammatory aneurysms remains obscure, but evidence presented above tends to confirm current theories of immunologically mediated inflammation.

7. In cases of inflammatory aneurysm, there is an acute phase protein response, confirming the presence of active inflammation. As part of this response, alpha-1-antitrypsin levels are raised non-specifically. This rise is appropriate and normal. The antitrypsin is phenotypically normal, and the rise is accompanied by an increase in anti-elastase activity, suggesting that antitrypsin function is also normal. This evidence presented tends to refute theories of aneurysm formation based on the premise that antiprotease function is defective.

8. The presence of an inflammatory aneurysm is accompanied by a rise in peripheral blood elastase complex. In

combination with some initial results suggesting a peripheral rise in elastin fragments, there is reason to believe that active ongoing elastin destruction is present.

9. It is possible that destructive processes in the wall of inflammatory aortic aneurysms may be the basis for a mechanism which tends to perpetuate the inflammation.

Scope for further work

Although this work has demonstrated some of the features accompanying inflammatory aneurysms, the root cause of this interesting disease is still unknown.

If the immunological mechanisms of ætiology outlined above are accepted as the most likely, the nature of the responsible antigen, although thought to be oxidised lipoprotein products of atheroma (*ceroid*) is still obscure. Isolation and tissue culture of lymphocytes from inflammatory aneurysm wall might be expected to provide the answer to at least some of the questions surrounding ætiology. If immunoglobulin products could be isolated from these cells and characterised, the nature of the antigen may be better understood. With this understanding, it may even be possible to prevent the development of inflammatory change by immunological manipulation.

Although evidence of increased elastin destruction in inflammatory aneurysms is presented, there is still the question of which comes first - the

inflammatory change or the elastin destruction? Certainly simple aneurysms show histological evidence of elastin destruction, and it may simply be that elastin destruction in inflammatory aneurysms is non-specifically increased secondary to mural inflammation.

Tilson has already produced some experimental evidence that aneurysm growth can be retarded using chemotherapy. His confirmation of Bouceks finding that beta-blockers inhibit aneurysm formation in an animal model by affecting collagen cross-linking suggests the possibility of prophylaxis in human aneurysm disease.[267,268] Small retrospective studies in this field have been encouraging.[269] In the light of these initial results it is possible that specific chemotherapy for small aneurysms will become available, which retards growth and prevents rupture. Such agents would also be expected to prevent inflammatory change.

The clinical staging of aneurysms and diagnosis of inflammatory change remains a problem. Magnetic resonance imaging has shown itself to be highly accurate in this respect, and has the advantage of being completely non-invasive. It is likely that M.R.I. will become at least as freely available as C.T. is currently, and as it provides all of the information necessary for safe surgical repair, it is probable that it will become the imaging method of choice for aneurysm investigation.

Further work is necessary to determine the true nature of the laminations seen on M.R. images of inflammatory aneurysm wall. Although it appears to correspond to laminations seen on low-power light microscopy sections of wall, a more detailed appraisal has to be made before this is completely understood.

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Appendix 1. Photocopy of the first paper to describe inflammatory aneurysm. The author was T.G.I. James, and the article appeared in the British Journal of Urology in 1935 (see reference 96).

The sequence of events became, of course, quite clear. I can find no reference to a similar case in the literature in my possession.

My thanks are due to Dr. W. L. Webb, Director of Medical Services, Zanzibar, for permission to publish this note.

URÆMIA DUE TO ANEURYSM OF THE ABDOMINAL AORTA

By T. G. Illtyd James, M.Ch., F.R.C.S.,
London.

The specimen here described was found at autopsy in a woman of fifty-two who had been admitted with symptoms of uræmia. A detailed history was not available, and as the patient was stuporose on admission she was unable to co-operate in examination. A large fixed mass with an expansile pulsation was found in the abdomen extending from the ensiform process down to a level just below the umbilicus. A catheter specimen of urine yielded a specific gravity of 1010 with some albuminuria. The clinical condition of the patient was that of advanced uræmia.

The patient died the day following admission and the investigations were therefore incomplete. It was ascertained that she had been under treatment four years before at another hospital for pain in the back with a swelling in the abdomen. A diagnosis of aneurysm of the abdominal aorta had been made. At that time the Wassermann and Sigma reactions were negative and the blood pressure $\frac{140}{90}$.

An autopsy revealed a generalised atheroma of the arteries. The abdominal aorta presented an unruptured aneurysm extending from the level of the bifurcation up to the origin of the superior mesenteric artery. The wall of the aneurysm was very thick, in some parts nearly 1 inch. The vertebral bodies were deeply eroded. The kidneys were reduced to one-third the normal size and presented the macroscopic and microscopic appearances of chronic interstitial nephritis. The renal arteries ran obliquely upwards in the wall of the aneurysm and would admit only the finest probe. The ureters were intimately adherent to the wall of the aneurysm, considerably narrowed and flattened, but not completely obstructed. The calices were not dilated.

The renal atrophy would appear to be due to a combination of pressure on the renal arteries and ureters by the gradual increase in the size of the aneurysm.

Appendix 2. Letter sent to patients before undergoing CT & MRI scans.

Tel 230000, ext 2836.

Vascular Studies Unit,
Level 7,
Bristol Royal Infirmary,
Marlborough St.,
Bristol.

Dear ,

You are on our waiting list to come into hospital for repair of your aortic aneurysm, the swelling in the main blood vessel in your abdomen. There ar a few investigations which should be carried out before you come into hospital, to give us important information about the size and location of your aneurysm. You may already have had an ultrasound scan, but there are two more scans which I will arrange for you to have. The first is a CT scan which will be performed at Bristol Royal Infirmary, and the second is an M.R.I. scan, which must be performed at Frenchay Hospital.

Neither of these scans are uncomfortable or painful, but it is important that you let me know if you have any of the following things, as they affect the M.R.I. scan:

A heart pacemaker

An artificial joint replacement

Any other bits of metal inside you, such as shrapnel, bullets etc.

If you have any questions about either of these scans, or require help to get to the hospitals, please phone me at the number above. If I am not available, please leave a message, and I will phone you back.

Yours sincerely,

W. G. TENNANT

Appendix 3. Clinical Questionnaire used to collect part of prospective data.

Clinical Questionnaire.

NAME D.O.B. HOSP NO.

OP DATE OPERATION

DISEASE TYPE OCCLUSIVE ANEURYSM INF ATH

SMOKER NO YES COAD?

DIABETES NO YES DIET TABS INSU NIL

PAST M.I. NO YES MEDS? CABG?

ANGINA NO YES ON LEVEL? HILLS?

PAST C.V.A. NO YES RESIDUA?

CLAUDICANT NO YES YARDS YEARS

REST PAIN NO YES NIGHT DAY

LIVER FT'S NORMAL ABNORMAL HOW?

U & Es NORMAL ABNORMAL HOW?

OVERT INFECTION NO YES WHERE?

SYMPTOMS Weight

Pain

Lassitude

Mass

CHOLESTEROL LIPIDS GLUCOSE

PULSES B.P.

KNOWN OR APPARENT HYPERTENSION?

MEDICATIONS

INVESTIGATIONS U/S CT MRI ANGIO BLOODS SPECIMEN

POST-OP COMPLICATIONS

r J Vasc Surg 6, 199–203 (1992)

Metabolic Activity in Inflammatory and Non-inflammatory Aneurysms of the Abdominal Aorta*

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Vascular Studies Unit, Bristol Royal Infirmary, Marlborough Street, Bristol BS2 8HW, U.K.

Inflammatory aneurysms of the abdominal aorta (IAA) comprise 10–15% of all aortic aneurysms (AA) but their aetiology and pathogenesis are obscure. Destruction of mural elastin is a prominent feature of IAA, and both increased elastolysis and decreased inhibition of elastolysis have been implicated. In order to study these factors, we have examined the peripheral blood of three groups of patients; 15 with inflammatory aortic aneurysms (IAA), 61 with simple aortic aneurysms (SAA) and 35 with aorto-iliac occlusive disease (OD). In all cases, alpha-1-anti-trypsin (A-1-AT), alpha-2-macroglobulin (A-2-MG), elastase inhibitory activity (E.I.A.), elastase-anti-trypsin complex, C-reactive protein (CRP), caeruloplasmin (CP) and plasma viscosity were measured. Patients with IAA had a significantly higher plasma viscosity (Mann–Whitney, $p < 0.05$), E.I.A. (Mann–Whitney, $p < 0.01$) and levels of A-1-AT, CRP, CP and elastase/anti-trypsin complex (Mann–Whitney, all $p < 0.05$) than patients in the other two groups. There was no difference in the levels of A-2-MG between any of the groups. This study refutes the theory that reduced inhibition of elastase activity predisposes to the formation of SAA. In patients with IAA, raised marker levels indicate ongoing destruction of elastin, and suggest a difference in pathogenesis between IAA and SAA. The study also suggests that IAA are highly active metabolically, as opposed to the more degenerative SAA.

Key Words: Aortic aneurysm; Inflammatory aneurysm; Alpha-1-anti-trypsin; Neutrophil elastase.

Introduction

e possible causes of aortic aneurysms include etabolic changes leading to destruction of elastin d/or collagen in the aortic wall. As these are the o major structural proteins present, a decrease in her could easily be responsible for aneurysmal dila-n. From the studies of Dobrin, however, destruc-n of elastin rather than collagen is more likely to d to aneurysm formation.[1] While there is histologi- evidence of elastin destruction in simple aneur-n disease of the aorta, it is most marked in the so led "inflammatory" variant.[2]

Inflammatory aortic aneurysms (IAA) are a sub- making up 10–15% of all aortic aneurysms[3,4] and e frequently recognisable macroscopically at oper-on, being encased in a thick white "inflammatory" at of fibrous tissue covering the anterior and lateral rfaces. In some cases, the fibrous tissue can extend into the retroperitoneum to encase adjacent hollow organs, such as the inferior vena cava, duodenum and ureters.[5,6] These aneurysms are notoriously difficult to detect preoperatively, and repair is more difficult with a high operative mortality.[5]

An increase in elastase activity and a decrease in elastase inhibition have both been implicated in the formation of aortic aneurysms.[7] Because of the histological differences between simple and inflammatory aneurysms, it is reasonable to believe that there may be differences in elastin metabolism, which might be especially true if the inflammation in IAA is active, as is likely from the clinical appearances and natural history of the disease.

This study aimed to investigate indirect evidence of inflammatory activity and elastin metabolism in the peripheral blood of patients with aneurysmal disease (of both simple and inflammatory types), and aorto-iliac occlusive disease.

Materials and Methods

The following patient groups were studied.

Group 1: 15 patients with IAA, mean age 67

resented at the 5th Annual Meeting of the European Society for scular Surgery, Warsaw, September 1991.

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years. There were two females in this group. The inflammatory nature of the aneurysms was detected preoperatively by radiological investigations, and confirmed at operation by the typical appearances. Subsequent histological diagnosis was made in all cases in accordance with previously published criteria.[8]

Group 2: 61 patients with simple, non-inflammatory aneurysms of the aorta (SAA), mean age 72 years. There were 11 females in this group. Diagnosis was made on similar criteria to group 1.

Group 3: 35 patients with severe aorto-iliac occlusive disease (OD) requiring aortic bifurcation grafting. This group included five females, and had a mean age of 64 years.

Specific exclusions were made from the study in cases with malignant or connective tissue disease, and infective illnesses. A number of biochemical assays were performed on the peripheral blood of each patient, and are detailed below.

Collection of plasma and serum samples

Fasting samples of blood were collected preoperatively from all patients using the "Vacutainer" system. Whole blood was sent to the Hospital Haematology Laboratory for plasma viscosity estimation and the remainder immediately centrifuged and separated into 1-ml aliquots of plasma and serum. Samples were stored at −80°C until used in the assays.

Acute phase proteins

The following acute phase proteins were measured: C-reactive protein (CRP), caeruloplasmin (CP), alpha-1-anti-trypsin (A-1-AT) and alpha-2-macroglobulin (A-2-MG). A standard immunoturbidimetry technique on a computer orientated random access immunoturbidometer (COBAS MIRA, F. Hoffman-LaRoche, Basle, Switzerland) was used. For each assay run, one sample in 10 was a control specimen of known antigen content. Their absorbance and concentration was used to validate the standard absorbance curve for normal laboratory use. Calibration of the analyser is maintained to give a coefficient of variation for protein assays of 2–3%. The specific antibodies and calibrators for each assay are shown below.

Serum C-reactive protein assay. Antibody: PRU Atlantic Antibodies anti-C-reactive protein; calibrator: Atlantic Antibodies Calibrator 7 in six dilutions in bovi serum albumin; controls: Atlantic Antibodies Calibr tor 7 prediluted 1:2 and 1:4 with bovine serum alb min to bring into range of standard curve.

Caeuloplasmin assay. Antibody: Atlantic Antibodi anti-caeruloplasmin; calibrator: Atlantic Antibodi Calibrator 1 in six serial dilutions; controls: Atlan Antibodies Calibrator 3, Behring protein standa plasma.

Serum alpha-1-anti-trypsin assay. Antibody: Atlan Antibodies anti alpha-1-anti-trypsin; calibrator: Atla tic Antibodies Calibrator 3, in six serial dilutio controls: Atlantic Antibodies Calibrator 1, SPSO1.

Serum alpha-2-macroglobulin assay. Antibody: Atlan Antibodies anti-alpha-2-macroglobulin; calibrat Atlantic Antibodies Calibrator 1 in six serial dilutio controls: Atlantic Antibodies Calibrator 3, Behri standard human serum.

Elastase–anti-trypsin complex

These were measured using the enzyme-link immunosorbent assay. A commercially available l (Merck Immunoassay, kit number 12589, 2h versio was suitable for polymorphonuclear leukocyte el tase. In this assay, plastic tubes coated with she antibody to neutrophil elastase are used. A standa volume of serum is added to the tube, and the co tained elastase–anti-trypsin complex is bound to t tube wall by its elastase moiety. The amount of bin ing is proportional to the amount of elastase–an trypsin complex present in the sample. The tube then washed, and a conjugate of anti-trypsin an body/alkaline phosphatase added which in tu binds to the anti-trypsin moiety of elastase–anti-try sin complex, forming a complex with an enzyma "tail" of alkaline phosphatase. After washing, t tube-bound enzyme activity is measured by its acti on 4-nitrophenyl phosphate, producing a colo change at 405 nm.[9]

All assays were carried out in duplica Standard solutions of known elastase/anti-tryps complex content were assayed in order to produc standard line, which was verified by assay of control serum. A computer based regression functi was used to calculate the values of unknown samp from their absorbance values. Inter-kit reproducibil was confirmed by assaying at least two of 22 anor mous samples in each kit and each anonymo sample was assayed at least twice.

ıstase inhibitory activity

ıe principle of this assay depends on the inhibitory ·wer of patients' serum on a standard elastase load. tients serum is added to a fixed amount of porcine ıstase, and incubated. During incubation, the ındard elastase load is inhibited to a degree pro-rtional to the amount of anti-elastase in the serum. ıe resultant serum/elastase mixture is then used to ɜest a fixed amount of elastase substrate, the more bstrate digested the less elastase inhibitor is esent in the patient's serum. Digestion of the sub-ate produces a colour change detectable by spec-ɔphotometry at 405 nm.

Calibration of the assay in our laboratory was rried out using plasma samples of known anti-'psin phenotype and inhibitor assay. The seven mples used for calibration were of mixed pheno-ɔes for A-1-AT, there being two Z types, two MZ ɔes one MS type, and two M types. Adequate sep-ıtion of phenotypes was obtained using the assay our laboratory following modification of the tech-ɟue. Three disparate known calibration samples ɔm the seven originally used in assay calibration ɜre also included in order to validate the "spread" results for unknown samples.

Final results from the assay of known elastase utions were then used to construct a standard rve and the results of the assay of the three known mples were plotted in order to confirm adequate ɔaration. Only if good separation was obtained was ɜ curve used to read unknown samples.

All the results were obtained by reading the sorbance of the samples on a computer controlled croplate spectrophotometer.

In practice, the principle elastase inhibitor ɜsent in human serum is A-1-AT. The assay there-·e essentially measures A-1-AT activity. Because of non-specific nature, however, it measures the ility of the entire serum to inhibit elastase. In this y, small differences in elastase inhibitory activity e to factors other than A-1-AT are also detected.[10]

Results

ıe results of this study are summarised in Tables 1 d 2 as means, with standard error in parentheses. all cases, the Mann–Whitney *U*-test was used to sess the statistical significance of differences be-·een groups.

C-reactive protein, CP and A-1-AT (Tables 1 and 2) were all significantly raised in the group of patients with IAA when compared to both SAA ($p < 0.04$ for all proteins) and OD ($p < 0.05$ for all proteins). Similar levels were found in patients with SAA and OD. There was no significant difference in levels of A-2-MG (Table 2) between groups. Plasma viscosity (Table 1) was raised in patients with IAA compared to SAA and OD ($p < 0.03$, Mann–Whitney, in both cases).

Table 1.

Disease type	CRP (mg l^{-1})	CP (g l^{-1})	Plasma viscosity
Inflammatory aneurysms	37.47 ± 11.01	0.45 ± 0.02	1.79 ± 0.05
Simple aneurysms	21.37 ± 3.1	0.40 ± 0.01	1.65 ± 0.01
Occlusive disease	18.05 ± 3.39	0.39 ± 0.02	1.64 ± 0.02

Results expressed as the mean ± S.E.

Table 2.

Disease type	A-1-AT (g l^{-1})	A-2-MG (g l^{-1})	Elastase–anti-trypsin complex (μg l^{-1})	Elastase inhibitory activity (%)
Inflammatory aneurysms	2.31 (0.13)	2.20 (0.13)	88.15 (12.74)	13.40 (0.98)
Simple aneurysms	1.94 (0.07)	2.47 (0.09)	59.69 (3.11)	21.35 (1.25)
Occlusive disease	1.88 (0.08)	2.46 (0.12)	61.04 (5.75)	19.47 (2.17)

The assay of elastase anti-trypsin complex revealed two very high values of 124 μg l^{-1} and 248 μg l^{-1} in the inflammatory group. The mean levels of elastase anti-trypsin complex were significantly higher in inflammatory aneurysms than simple aneurysms when these two values were included (Table 2, $p < 0.005$) and when they were excluded ($p < 0.005$).

Values for the elastase inhibitory assay (Table 2) were significantly lower in the IAA group than the SAA group ($p < 0.001$). The figures represent the amount of residual elastase activity remaining in the specimen following inhibition by the patient's plasma, consequently the lower the value, the more active is the patient's plasma in inhibiting the action of elastase. Once again, there was no difference between the group with SAA and those with OD.

None of the protein or proteinase assays either singly or in combination were predictive of the presence of an inflammatory aneurysm.

Discussion

Data on the role of elastases in the pathogenesis of aortic aneurysm disease are contradictory. Mechanisms have been proposed by a number of authors in which the causative defect is either a primary rise in elastase activity, or decrease in anti-elastase activity allowing a secondary rise in elastase activity.[7]

Similarly, little is known about the metabolic activity of inflammatory aneurysms, or its effect on the patient. Since it is likely that the presence of an aneurysm provokes the inflammatory reaction rather than vice versa, it is essential that the nature of the inflammatory response be clearly defined. The reason why only some aneurysms become inflamed is unknown, and fibrosis in established IAA appears to progress slowly with time. It is important to consider the possibility that highly active inflammation and tissue destruction are present, rather than inert "burnt out" fibrous tissue. If the inflammation is active, then it may be possible to detect it. If more is known of its precise nature, then more specific non-operative treatment may also be possible.

The levels of acute phase proteins in the peripheral blood of patients with SAA approximate to those seen in severe aorto-iliac OD bearing out the proposition that simple aneurysms represent a degenerative disease. Tissue destruction, although present, does not occur either at a rate or to a degree sufficient to induce a generalised response.

Within the wall of inflammatory aneurysms there is histological evidence of inflammation. The cellular picture is of 'chronic' inflammation with an abundance of lymphocytes, plasma cells and macrophages.[2] Together with the generally accepted theory that an aneurysm leads to fibrosis, the research of Parums and Mitchinson[11] suggests that inflammation is due to expression of an antigen through damaged medial tissue. This is certainly possible and might be expected to lead to the type of response actually seen in inflammatory aneurysms with the elaboration of fibrous tissue.

From the results of this study there is biochemical evidence to suggest that a generalised response to active inflammation is present in patients with an inflammatory aneurysm and raised peripheral blood levels of CRP, CP and A-1-AT are compatible with an immunologically mediated inflammatory process, and support theories of aetiology based on this premise.

Destruction of elastin in the aortic wall has been noted in both SA and IAA, but is more prominent in IAA. This could be a simple mechanical effect, or be due to continuing active destruction by elastases. Powell[12] has found increased levels of neutrophil elastase in the wall of inflammatory aneurysms a an assay of this enzyme has been used clinically monitoring the activity of both rheumatoid arthrit and inflammatory bowel disease.[13]

Assay of the neutrophil elastase complex in t study has suggested that levels are increased in t peripheral blood of patients with inflammat aneurysms compared with both SAA and OD. elastase levels are similar in SAA and OD, a prima increase in elastolysis is unlikely as a cause of SA For the same reason, it is unlikely that a prima increase in elastolysis is responsible for the inflamm tory disease. It is much more probable that increas elastolysis is a consequence rather than a cause of t inflammatory process. It may either be a part of t cellular/humoral response to inflammation, or it m be provoked by the production or exposure damaged elastin in the aortic wall. In general, neut phils are not a prominent feature of the cellular sponse in the wall of inflammatory aneurysms, a so it is curious that peripheral blood levels are raise There remains the possibility that another cell ty (e.g. macrophages or lymphocytes) may produ elastase antigenically indistinguishable from that p duced by neutrophils.

In this respect, one of the acute phase protein A-1-AT deserves further consideration. As the prin pal inhibitor of neutrophil elastase in the periphe blood, A-1-AT has been implicated in the aetiology simple aneurysms. Such theories have their basis the interaction between cigarette smoking and an trypsin. Oxidants in cigarette smoke are capable oxidising methionine residues on A-1-AT, resulti in its inactivation[14] which is thought to be the cau of emphysema in smokers. The mechanism whi had been proposed in aneurysm disease is that de ciencies in anti-trypsin levels or function ha allowed an increased, uninhibited action of elasta leading to aneurysm formation. The high incidence smoking in all forms of vascular disease preclud this as a specific cause of aneurysm disease, but might be possible that some other cause of reduc anti-trypsin activity could result in the same mecha ism of aneurysm formation. In the light of the resu of this study, however, this seems unlikely, as t total assay and activity of A-1-AT are no different patients with simple aneurysms and those w severe aorto-iliac OD.

Parums and Mitchinson[11] propose a mechani for inflammatory change in which antigen extruc through a severely damaged aortic media provoki inflammation. If IAA represents an extreme form degenerative disease, the assay or function of A-1-A activity should be more severely defective in the

ses. On the contrary, A-1-AT levels are higher in tablished inflammatory disease than in the other 'o groups, and this increase is accompanied by an crease in total anti-elastase activity. This is simply e non-specific "damage limitation" action of A-1-Γ in its role as an acute phase protein, and occurring a response to inflammation. It cannot be regarded any way as specific to inflammatory aneurysm.

Even though this study is of patients with estab-hed disease, and the rise in A-1-AT is non-specific, e results presented suggest that defective anti-otease function does not predispose to the forma-ɔn of either simple or inflammatory aneurysms. If it ɜre to do so, then some abnormality in the acute ıase response or anti-protease function might be pected to be present. No evidence has been found support this.

In conclusion, in cases of simple non-inflamma-ry aortic aneurysm, there is no systemic evidence of sue destruction or inflammation and there is no ɔrease in elastase activity, or decrease in anti-otease function. Where inflammatory change is esent, there is an acute phase protein response, dicating active, ongoing tissue destruction and lammation. This is accompanied by rises in both ıstase inhibitory activity in the peripheral blood, d neutrophil elastase complex, both of which are ely to be secondary to the inflammatory process her than its cause.

These results refute the theory that progress ɔm simple to inflammatory aneurysm is due to ɜatly decreased anti-protease function. Once estab-hed, the inflammatory process may perpetuate elf through local elastase production, and destruc-n of aortic media, so provoking the cellular ɜponse.

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Identifying Total Carotid Occlusion with Colour Flow Duplex Scanning*

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A major limitation of conventional duplex scanning is its inability reliably to differentiate severe stenosis from total occlusion of the internal carotid artery (ICA). Colour flow duplex scanning (CFS) facilitates the identification of internal and external carotid arteries, enables simultaneous evaluation of flow in multiple vessels in longitudinal and transverse views, and allows more accurate assessment of very low Doppler-shift frequencies with new "slow-flow" software technology. From July 1987 to January 1991, 9731 ICAs (4866 patients) were evaluated with CFS. Arteriography was performed in 483 of these patients (959 ICAs), and the results of the two studies were compared. Colour flow scanning was highly accurate in differentiating total occlusion from carotid stenosis. Eighty-two of 87 totally occluded ICAs were detected (sensitivity 94%) and 873 of 878 patent arteries were properly identified (specificity 99%). Positive and negative predictive values were 93 and 99%, respectively. False positive results (n = 6) were due to interpreter error (n = 4) and poor scanning technique (n = 2). All false negative results (n = 5) were the result of interpreter error. During the last 24 months of the study, no false positive or false negative results were detected, giving an accuracy of 100%. We conclude that CFS offers distinct advantages in the diagnosis of carotid occlusion, thereby overcoming the limitations of conventional duplex scanning in distinguishing total occlusion of the ICA from less severe disease, and is the method of choice for evaluating the carotid bifurcation.

Key Words: Colour flow scanning; Duplex scanning; Total carotid occlusion.

Introduction

Prevention of stroke and preservation of neurological function are the goals of treating extracranial carotid occlusive disease. Appropriate therapy depends on accurate identification of an internal carotid artery (ICA) lesion amenable to direct surgical correction. Recent clinical trials, both in Europe and in North America, have shown conclusively that carotid endarterectomy (CEA) substantially reduces the risk of stroke at 2–3 years compared with "optimal" medical therapy in symptomatic patients with 70–99% diameter stenoses of the internal carotid artery.[1,2] Although the results of similar trials in asymptomatic patients have yet to be published, preliminary data suggest that the outcome in terms of stroke reduction is similar.[3] On the other hand, surgical treatment of total carotid occlusion is hazardous and ineffecti except in rare cases when the occlusion is thrombo and treatment can be instituted immediate Methods for evaluating the carotid artery mu therefore, be able to distinguish between severe ste osis and total occlusion. During the past decade, co ventional duplex scanning has become the diagnos procedure of choice for the non-invasive evaluati of the extracranial ICA. While many centres ha reported excellent results documenting the accura of duplex scanning,[4–7] a major limitation exists in inability reliably to differentiate severe stenosis fr total occlusion of the ICA.[8,9]

Colour flow duplex scanning (CFS) represe the most recent technological development in no invasive vascular evaluation. Although seve reports have confirmed the accuracy of this modal for detecting disease of the ICA,[10–13] the only pa specifically addressing the issue of total ICA occ sion did not compare the results of CFS to a "g standard".[14] To determine whether CFS overcon the limitations of conventional duplex scanning in differentiation of severe carotid stenosis from to

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